LAMK Lahden ammattikorkeakoulu
Lahti University of Applied Sciences

BPA performance (TOC, BOD and toxicity) during photo-Fenton reaction

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KAISTO, TIINA:

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ABSTRACT

This project was a part of the author's four months Erasmus+ exchange studies at Universitat Politècnica de Catalunya Barcelonatech (UPC, Spain) via Lahti University of Applied Sciences (Lahti UAS, Finland). The project was carried out in the chemical engineering department of UPC at the College of Industrial Engineering of Barcelona (EUETIB) under the guidance of professor Montserrat Pérez-Moya. Previously in 2013, in EUETIB, Marina Navarro had studied the effects of the photo-Fenton process on bisfenol-A (BPA). This study was implemented based on Navarro's results.

BPA is a chemical used in plastic production. It is considered as an endocrine interrupter, although research results about the health risks of BPA are under scientific debate. Advanced oxidation processes (AOPs) are methods to treat wastewater, photo-Fenton reaction being one of them. In the photo-Fenton reaction, hydrogen peroxide and iron(II) are used as reagents together with UV- light irradiation. Based on previous studies, the by-products which appear during the photo-Fenton process, may be even more toxic than BPA itself, effecting on wastewater treatment systems. In this study, the main idea was to evaluate the evolution of toxicity during the photo-Fenton process, and to study the biodegradability of BPA and the end products of the photo-Fenton process.

The theory part presents the latest information about BPA according to the recent risk assessment of the EU. Chapter 3 includes the analytical and Chapter 4 the experimental methods. The concentrations of reagents being variables with BPA concentration of 30mg/L, preliminary experiments and 14 experiments were implemented. TOC, H_2O_2 and toxicity were analyzed during the experiments and BOD_5 from the BPA solutions and the end products of the experiments. Biodegradability was evaluated using the BOD/COD ratio.

As a conclusion, a mineralization rate of over 80% was achieved, when the H_2O_2 concentration was 100.63 mg/L and at least 4mg/L Fe(II) was used. BPA of 30 mg/L was found not biodegradable, 20 mg/L was partially biodegradable and the lower concentrations were totally biodegradable. After the photo-Fenton treatment, all the solutions were at least partially biodegradable. The results seem to indicate that in such low concentrations neither BPA nor the by-products were toxic for the bacteria, but were used as a source of aliment instead. LD_{50} of BPA for this bacteria was 40000 mg/L. The used method for toxicity analysis was viewed critically. Because of the different effects due to the bacteria strain used, cautiousness is needed if both biodegradability and toxicity are evaluated based on bacterial methods. Combining AOPs and biodegradation could be one solution for the removal of pollutants, such as BPA, from the wastewater, which requires further studies.

Key words: BPA**,** bisphenol-A, biodegradability, photo-Fenton, toxicity, degradation, mineralization, by-product, intermediate product, AOP

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TIIVISTELMÄ

Tämä projekti on toteutettu osana Lahden ammattikorkeakoulun (LAMK) Master-tutkintoon liittyvää kolmen kuukauden Erasmus+ -vaihto-opiskelua Espanjassa (Universitat Politècnica de Catalunya Barcelonatech, UPC). Projekti tehtiin UPC:n kemiantekniikan laitoksella professori Montserrat Pérez-Moyan ohjaamana (College of Industrial Engineering of Barcelona, EUETIB). Vuonna 2013 Marina Navarro oli tutkinut EUETIB:ssa photo-Fenton- reaktion vaikutusta bisfenoli-A:han (BPA). Tämä tutkimus perustuu Navarron tuloksiin ja on jatkoa aiempaan projektiin.

BPA on muoviteollisuudessa käytetty hormonitoimintaa häiritsevä yhdiste, vaikkakaan BPA:n terveyshaitoista tehtyjen tutkimusten tuloksista ei olla yksimielisiä. Jäteveden puhdistamisessa käytetyistä AOP-menetelmistä (Advanced Oxidation Process) tutkittavana oli photo-Fenton reaktio. Photo-Fenton reaktiossa vetyperoksidi ja rauta(II) reagoivat UV-säteilyn kanssa. Aiemmat tutkimukset osoittavat, että reaktion aikana muodostuvat sivutuotteet voivat olla jopa haitallisempia kuin BPA itsessään. Tämän tutkimuksen päätarkoituksena on arvioida toksisuuden muuttumista photo-Fenton reaktion aikana sekä tutkia BPA:n ja photo-Fenton reaktion lopputuotteen biohajoavuutta.

Teoriaosuudessa on kerrottu viimeisimmistä EU:n riskinarvioinnista ja luokittelusta BPA:ta koskien sekä esitetty taustaa tutkimukselle. Analyyttiset menetelmät on esitelty kappaleessa 3 ja kokeellinen tutkimusmenetelmä kappaleessa 4. Alustavat photo-Fenton-kokeet sekä 14 varsinaista koetta toteutettiin BPA-liuoksella (30 mg/L), missä reagenssien pitoisuudet olivat muuttujina. TOC, H_2O_2 ja toksisuus analysoitiin kokeen aikana otetuista näytteistä ja BOD_5 BPA-liuoksista sekä kokeen lopputuotteesta. Biohajoavuutta arvioitiin BOD/COD-suhteella.

Tutkimuksessa havaittiin, että yli 80% mineralisaatio saavutettiin käyttämällä photo-Fenton reaktiossa 100.63mg/L H_2O_2 ja vähintään 4mg/L Fe(II). 30 mg/L-pitoinen BPA-liuos määritettiin biohajoamattomaksi, 20 mg/L osittain biohajoavaksi ja sitä matalammat pitoisuudet täysin biohajoaviksi. Photo-Fenton reaktiolla käsitellyt näyteliuokset olivat kaikki vähintäänkin osittain biohajoavia. Tulosten mukaan tämän tutkimuksen verrattain alhaiset BPA:n ja sivutuotteiden pitoisuudet eivät olleet toksisia käytetyille bakteereille, vaan päinvastoin bakteerit pystyivät käyttämään niitä ravintona. Käytetyille bakteereille BPA:n LD_{50} -arvon todettiin olevan 40000 mg/L.

Toksisuuden määrittämiseen käytettyä menetelmää on tarkasteltu kriittisesti. Vaikutukset bakteereihin eroavat huomattavasti eri bakteerikannoilla. Kantojen väliset eroavaisuudet voivat vääristää tuloksia, joten tulosten soveltamisessa tulee olla varovainen kun arvioidaan sekä biohajoavuutta että toksisuutta bakteereja hyödyntäen. AOP-menetelmien ja bakteerien avulla tapahtuvan

biohajottamisen yhdistämistä voi pitää varteenotettavana mahdollisuutena BPA:n kaltaisten aineiden poistamiseksi jätevedestä. Menetelmien optimointi on kiinnostava tutkimusaihe niin BPA:n kuin muidenkin haitallisten orgaanisten aineiden poistamiseksi jätevedestä.

Asiasanat: BPA, bisfenoli-A, photo-Fenton, biohajoaminen, hajoaminen, mineralisaatio, myrkyllisyys, sivutuotteet, jäteveden käsittely

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ABBREVIATIONS AND MOST OFTEN USED CHEMICAL FORMULAS

AOP	Advanced Oxidation Process, techniques used for wastewater treatment
BMDL	Benchmark dose (Lower Confidence Limit)
BOD_n	Biological Oxygen Demand, n indicates the number of days used for the test, usually BOD_5, but in some cases BOD_7 or some other duration
BPA	Bisfenol-A, $C_{15}H_{16}O_2$, which is also called 2,2-bis(4-hydroxyphenyl) propane or 4,4'-Isopropylidenediphenol (CAS no. 80-05-7), an endocrine disruptor, chemical used e.g. in plastic industry
BPS	Bisfenol-S, can be used as substitute for BPA
COD	Chemical Oxygen Demand, BOD/COD relation indicates the biodegradability of the substance (= water quality)
DO	Dissolved Oxygen
DOC	Dissolved organic carbon
DOE	Design of the Experiment
EC	European Commission
EC_{50}	Half maximal effetive concentration, concentration of a toxicant which induces a response halfway between the baseline and maximum after a specified exposure time
ECHA	European Chemicals Agency
EDC	Endocrine disrupting chemical
EDDS	Ethylenediamine-*N*,*N*′-disuccinic acid complex
EFSA	European Food Safety Authority
EUETIB	College of Industrial Engineering of Barcelona (one school of UPC), Spain
EU-RAR	European Union Risk Assessment Report
EUSES	European Union System for the Evaluation of Substances
Fe^{2+}, Fe(II)	Iron(II)-ion, in this experiment the compound used as reagent was iron(II) sulphate, $FeSO_4 \times 7H_2O$
GGA	Glutamic acid and Glucose solution, used here as aliment in BOD tests

HED	Human equivalent dose
H_2O_2	Hydrogen Peroxide, another reagent used in this experiment
H_2SO_4	Sulphuric acid (a strong acid)
LC	Lethal concentration, which is the concentration of a substance in an environmental medium that causes death following a certain period of exposure.
LD	Lethal dose, concentration of a substance or physical agent (e.g. radiation) that causes death when taken into the body
LD_{50}	Lethal dose, the dose when 50 % of the population dies (mice, bacteria etc.) after exposed to the chemical
LOAEL	(Lowest Observed Adverse Effect Level) is the lowest concentration or amount of a substance (dose), found by experiment or observation, which causes adverse effect on morphology, functional capacity, growth, development, or life span of the target organisms distinguishable from normal (control) organisms of the same species and strain under defined conditions of exposure.
Lahti UAS	Lahti University of Applied Sciences, Finland
NaOH	Sodium hydroxide (a strong base)
NOAEL	(No Observed Adverse Effect Level) is the greatest concentration or amount of a substance found by experiment or observation, which does not cause detectable adverse alteration of morphology, functional capacity, growth, development, or life span of the target organism under defined conditions of exposure.
·OH	Hydroxyl radical, which can break toxic and persistent compounds
OECD	Organisation for Economic Co-operation and Development
PBT	Persistent (a degradation half-life in the freshwater environment >40 days, or freshwater sediment >120 days, or marine water >60 days or marine sediment >180 days, or soil >120 days), bio accumulative and toxic, REACH-definition
PNEC	Predicted no-effect concentration
RAR	Risk assessment report (e.g. EU-RAR)
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals, chemicals policy of the EU

SD	Standard Deviation
SCF	Seed Control Factor, used in BOD test calculations
TDI	Tolerable daily intake is the estimated quantity of a chemical substance that can be ingested daily over a lifetime without poising a significant risk to health. TDIs are expressed by body weight, usually in mg or µg per kg of body weight and per day in the case of repeated exposure. t-TDI = temporary TDI
TOC	Total Organic Carbon, quantity of OC in the substance. The value can be used to calculate theoretical COD
UPC	Universitat Politècnica de Catalunya Barcelonatech, Spain
vPvB	Very persistent (a degradation half-life in freshwater or marine water >60 days or sediment >180 days, or soil >180 days) and very bio accumulative, REACH-definition
WHO	World Health Organisation

1 INTRODUCTION

1.1 Motivation

This project was a part of a four month Erasmus+ student exchange at Universitat Politècnica de Catalunya Barcelonatech (UPC). The project was carried out at the chemical engineering department at the College of Industrial Engineering of Barcelona (EUETIB) under the guidance of professor Montserrat Pérez-Moya. The toxicity analyses were performed in the laboratory of biochemistry (Escola Tècnica Superior d'Enginyeria Industrial de Barcelona/ETSEIB) together with Professor Luis Javier del Valle. The sending university was Lahti University of Applied Sciences (Lahti UAS, Finland), where the author was studying in the Master's Degree Programme in Environmental Technology.

1.2 Previous research

In UPC/EUETIB there has been research considering the photo-Fenton process on different substances. Previously, in 2013, there had been a project, where Marina Navarro had studied the effects of the photo-Fenton process on bisphenol-A (BPA).

Advanced oxidation processes (AOPs), such as Fenton and photo-Fenton, are considered as appropriate methods for treating persistent organic pollutants. In Navarro's study, H_2O_2 and Fe(II) initial concentrations were studied to evaluate the mineralization rate and their influence on the treatment performance. Samples were produced (BPA = 30mg/L) and the other variables were fixed (pH 3, temperature 25ºC, container = 0,5L, UV light source). The progress of the reaction was monitored, total organic carbon (TOC) measured, and the reaction rate (k) and the maximum conversion ξ^{max} were calculated by a pseudo first-order model to compare the results obtained experimentally. Among the photo-Fenton assays, the conditions providing the highest k to ξ^{max} were determined with relationships (BPA/ H_2O_2/ Fe(II)) 1,00/5,36/0,37; 1,00/5,36/0,25 and 1,00/6,70/0,33. (Navarro 2013, 9.)

Navarro concluded that under optimal concentrations of reagents, BPA is degraded in a few minutes but by-products occur. Previously, Katsumata identified six intermediates during the photo-Fenton process implemented on BPA and also concluded the possibility of still unidentified intermediates. (Katsumata 2004; Navarro 2013.)

1.3 The aim of the research

The purpose of this study is to continue previous research in order to find out the best possible conditions for the photo-Fenton reaction to remove BPA from the water. Because the basic idea is to clean for example industrial wastewater, it would be important that the treated water does not include other toxic or hazardous compounds. Treating BPA with the photo-Fenton process, it is possible that some by-products are even more toxic than BPA itself (Katsumata 2004, Oller 2011). After the photo-Fenton process, the water should not contain anything that disturbs the traditional wastewater treatment process or goes through it. At the end, the quality of the water should meet the environmental requirements. This means that the water should be biodegradable and not toxic for humans or the environment.

In Navarro's study (2013) it was found out that BPA disappears during the first 10 minutes of the photo-Fenton process. Furthermore, the amount of total organic carbon (TOC) is reducing slower and can be followed by analyzing the TOC of the samples during the photo-Fenton process. In this research, the aim was to find out if toxic by-products exist during or at the end of the process, and to specify at which point or in which conditions they will occur. The available toxicity method was not the best possible, but also learning these techniques was one aim of the study. The best possible conditions (amount of Fe^{2+} and H_2O_2) where the end product will be biodegradable were also determined. The aim was to find the best conditions for the process, and minimize the need and the costs of reagents in a larger scale.

Research questions:

1. Is the photo-Fenton treatment suitable to eliminate BPA and reduce the BPA by-products?

2. What are the best amount of reagents (H_2O_2 and Fe(II)) for photo-Fenton process in order to

    i) remove the BPA and the by-products?
    ii) have a good rate of BPA and by-products degradation?
    iii) ensure that the end product/s is/are biodegradable and not toxic?

3. In which conditions (quantity of iron and hydrogen peroxide) the end solution of the photo-Fenton experiment is biodegradable? Is BPA biodegradable in studied concentrations?

4. Are the by-products of photo-Fenton treatment or BPA itself toxic for the bacteria used? Confirmation of the LD50of BPA.

1.4 Methods

In previous research, it was determined that the photo-Fenton reaction with UV light was clearly more efficient than without light. Based on Navarro's (2013) research, the guidelines for this study could be determined considering the amounts of iron (Fe^{2+}) and hydrogen peroxide (H_2O_2) in the photo-Fenton experiment. In order to answer the research questions, three analysing methods were used to analyse the results of the photo-Fenton experiment: total organic carbon (TOC), biodegradability (BOD) and toxicity. Defining the design of the experiment (DOE) is explained later in Chapter 4.5. Briefly, 20 experiments were performed including the preliminary and the excluded ones. In addition, the solutions of known concentration of BPA (2.5; 5.0; 7.5; 10.0; 20.0; 30.0 mg/L) were made and the same analyses as for the end products of the experiments.

The experiments of the photo-Fenton treatment (deionised water, 30 mg/L BPA) were implemented in stable conditions, where pH and temperature

were monitored and adjusted if needed. The variables (Fe^{2+} and H_2O_2) were changed according to the design of the experiment (DOE) and samples taken in 5, 10, 20, 30, 45, 60, 75 and 90 minutes. At first, it was planned to take 60-minute experiments, but DOE was adjusted when preliminary experiments indicated that 90 minutes is needed. In order to be sure that all H_2O_2 was consumed, the absorbance was measured from the samples using a spectrophotometer. TOC was measured in each sample using a TOC analyzer (Shimadzhu TOC-$V_{CSH/CSN}$), which gives the amounts of organic and inorganic carbon. From each sample, 4,5 mL were stored in the freezer for the toxicity analyses. The solution left after the experiment was also stored in the freezer for the BOD analysis to be later.

The biodegradability of the end product was analyzed using the OxiDirect BOD System (Lovibond). BOD_5 was measured, but also the BOD_7 values were recorded in order to compare the results. BOD_5 is widely used, also in Spain, but in Finland the BOD_7 is used more often. To get the comparative results for the solutions of known concentrations of BPA and to define the final DOE, firstly the BOD analysis were to the BPA solutions of a known concentration (2.5; 5.0; 7.5; 10.0; 20.0 and 30.0 mg/L). These BOD analyses were using two volumes of the solution (360 mL and 428 mL) according to the possibilities of the equipment, and finally the volume of 360 mL was chosen for the experiments.

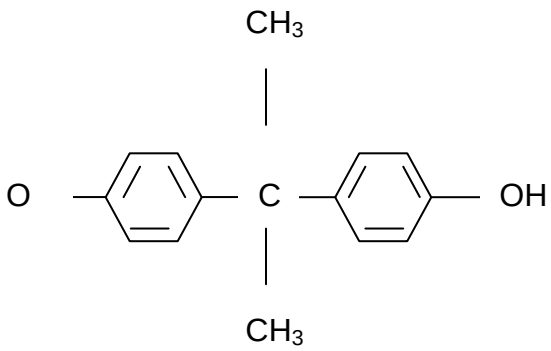
Toxicity was analyzed using two different bacteria, gram negative *Escherichia coli* and gram positive *Staphylococcus epidermidis*. The LD_{50} dose of BPA for both bacteria was also determined. However, the method used for analysing toxicity was not the best possible according to the literature and the qualities of BPA. After all, it was the available method during the project, and was used in order to familiarize the author for this kind of toxicity methods. In the beginning, plans were made to analyze also cytotoxicity, but due to the limited time and resources it was excluded from this study.

2 THEORY

The basic information about BPA, photo-Fenton reaction, and used analysing methods such as TOC, COD, BOD and toxicity is presented in the theory part. The latest information about BPA according to the EU risk assessment (2014) is also included.

2.1 Bisphenol-A (BPA)

2.2-bis(4-hydroxyphenyl)propane or 4.4'-Isopropylidenediphenol (CAS no. 80-05-7) is more commonly known as bisphenol-A (BPA), which is the name used throughout this report. The purity of BPA is 99-99.8 % depending on the manufacturer. The molecular formula of BPA is $C_{15}H_{16}O_2$ and molecular weight 228.29 g/mol. The solubility of bisphenol-A in pure water is 300±5 mg/L at 25.0±0.5 °C, with no significant variation over the pH range of 4 to 10. (EC 2003; 2010.)



(1)

The most important health effects of exposure to BPA are eye and respiratory tract irritation, skin sensitisation, repeat dose toxicity to the respiratory tract, effects on the liver and reproductive toxicity (EC 2003). The presence of BPA in food has been a special concern, since it is the primary route to human exposure. BPA is being released into the environment as well as into surface water during its manufacturing and by leaching from the final products.

Bisphenol-A is primarily used as an intermediate in the production of polycarbonate (75% of the total use in the EU 2005-2006), and epoxy

resins (17%) especially in the production and processing of PVC. It is also used for can coatings, food containers, thermal paper (0.2 % of the total use in the EU), tyre and brake fluid manufacture. The main route of environmental exposure of BPA is from the use in thermal paper and PVC industries. Updated information about emissions from production sites have been provided by the industry and published in the Environmental Risk Assessment of EU (Table 1). As one example, the amount of BPA

Table 1. Summary of environmental releases from bisfenol-A production sites in 2006. (EC 2010, 10.)

Site	Air		Effluent (After wastewater treatment)		Receiving water type and flow rate
	Measured levels	Release	Measured levels	Release	
BPA1	<0.2 mg/Nm³ (outlet) <0.5 µg/Nm³ (50 m from site)	<0.012 kg/day <4.4 kg/year	5.6 µg/l	0.06 kg/day 21 kg/year	Estuary 8.64 x 10^6 m³/day
BPA2	2.9 mg/Nm³ (outlet discontinuous) 0.1 µg/Nm³ (outlet)	0.00017 kg/day 0.0605 kg/year	3.13 µg/l	0.07 kg/day 27 kg/year	River 2.068 x 10^8 m³/day
BPA3	<1 mg/Nm³ (dust)	<1 kg/day (dust) <365 kg/year (dust)	~0.005 mg/l	0.31 kg/day 113 kg/year	Estuary 8.08 x 10^7 m³/day
BPA4		0.03 kg/day 9 kg/year		0.096 kg/day 35 kg/year	Estuary 2.49 x 10^7 m³/day
BPA5		1.58 kg/day (dust) 575 kg/year (dust)	Up to 45 µg/l (average 3.5 µg/l)	0.019 kg/day *6.8* kg/year	Estuary 6.1 x 10^8 m³/day
BPA 6	10 mg/Nm³ (dust)	0.08 kg/day (dust) 31.2 kg/year (dust)	Average 10 µg/l	0.072 kg/day 25.8 kg/year	Sea (dilution factor 100)

Values changed from the published risk assessment are underlined. The unit of Nm³ refers to air at standard temperature and pressure (the measurements may have been made originally with hotter air and so are corrected).

used in thermal paper in EU is 1700 tonnes and around 30% of this paper (510 tonnes) is estimated to enter the recycling streams. Together with the waste material of production, in total around 700 tonnes of BPA find its way to paper recycling sites each year. (EC 2010, 10; Ministry of Environment in Finland 2014, 15.)

In Finland, there is no production of BPA, and the main use is thermal paper used e.g. in shop receipts and lottery tickets. However, there are plenty of other uses of BPA in Finland, which are well listed in the draft version of the plan that the Ministry of Environment (2014) has composed for the toxic chemicals in the water environment (based on the issued decree 1022/2006, 12§). BPA is commonly found from the treated

wastewater as well as in the sludge of wastewater treatment plants and filtered water of the landfills. In Finland, the concentration of BPA in treated wastewater has been about 0.2-0.5 µg/L, which is the same level than in the other Nordic countries. Concentrations of the landfill waters vary a lot, but overall they are usually higher (< 0.05-300 µg/L). Some BPA can be found from the sludge, but it usually hardly stays in the sludge. BPA has also been found from the industrial wastewaters as well as normal household wastewater (1-2 µg/L) and storm water. (Ministry of Environment in Finland 2014, 15-19.)

BPA has been detected in 14 (out of 19) sample points of body of water in Finland. In Eurajoki, the concentrations have been higher than the predicted no-effect concentration (= PNEC, in inland waters 1.5-1.6 µg/L). In Mustionjoki and Porvoonjoki rivers, there have also been detected high values, but below PNEC. (Ministry of Environment in Finland 2014, 19.)

In the United States they have measured as high concentrations as 12 µg/L in surface water, 2.55 µg/L in ground water and 140 µg/L in freshwater sediment. These have raised concern, since the values are higher than the international PNEC values (0,175 to 1.6 µg/L, see Chapter 2.1.2). However, most environmental monitoring results show that the concentrations of BPA in water bodies are lower than 1 µg/L, although these results include uncertainties. (U.S.EPA 2010, 15.)

2.1.1 Health risks of BPA

The European Food Safety Authority (EFSA) decided in 2012 to make a full re-evaluation of the human risks associated with exposure to BPA through both dietary and non-dietary sources, such as thermal paper and dust. The reason for the re-evaluation was the huge number of published research studies in the recent years. Over 450 studies were reviewed, and national authorities and stakeholders were consulted. (EFSA 2014.)

As a result, EFSA recommended that the current daily intake (TDI, 50 µg/kg body weight/day, assessed in 2006) should be lowered to 5 µg/kg

bw/day and be set on a temporary basis. This means that TDI is temporary (t-TDI) until the results of ongoing research from the US National Toxicology Program can be incorporated in the evaluation. However, EFSA concluded that the health risk for consumers is low because the exposure to the chemical is well below the temporary TDI. The risk assessment of EFSA had public consultation in 2014. (EFSA 2014.)

However, there were many uncertainties at that point, and EFSA released the final results of the risk assessment in January 2015. After comprehensive re-evaluation of the exposure and toxicity of BPA, EFSA concluded that BPA poses no health risk to consumers of any age group, since the exposure from the diet or from a combination of sources (diet, dust, cosmetics and thermal paper) is three to four times lower than the safe TDI-level. The new safe level (t-TDI) was reduced to 4 µg/kg bw/day. EFSA concluded that high doses of BPA (more than 100 times the TDI) are likely to adversely affect the kidney and liver, and cause effects on mammary gland. Other health risks on reproductive, nervous, immune, metabolic and cardiovascular systems, as well as in the development of cancer were not considered likely at present, but they could not be excluded on the available evidence. (EFSA 2015a.) It should be noted that uncertainty in the exposure estimates for non-dietary sources was considered high because of the lack of supporting data. The uncertainty around dietary exposure was relatively low. (EFSA 2015c.)

BPA is often referred as an endocrine disruptor. However, it should be noted that despite the numerous scientific publications and research, these effects of BPA are still under scientific debate. Endocrine system is a network of glands which regulates and controls the release and levels of hormones in the body. It is a complex system, and scientific knowledge of it is still growing. Imbalances or of the endocrine system can result in well-known diseases, such as diabetes and obesity, infertility and certain types of cancer. Disruption of this system can also cause birth defects and learning disabilities. Chemicals that can interact or interfere the endocrine system are called endocrine active substances. The endocrine system is capable of adjusting and the effect is not always harmful. When the

interference leads to adverse effects, these substances are called endocrine disruptors. (EFSA 2015b,c.)

EFSA endorses the World Health Organization's (WHO) definition that a substance has to meet three criteria to be considered an endocrine disruptor; 1) the presence of an adverse effect, 2) the presence of endocrine activity and 3) a causal relationship between the two. In their 2015 opinion on BPA, EFSA's experts reviewed all literature on potential endocrine-related effects of BPA. They concluded that there is no single clearly-defined explanation that substantially completes scientific understanding of the potential effects of BPA in humans. Therefore, based on the WHO criteria, it is not possible to conclude that BPA is an endocrine disruptor. (EFSA 2015d.) Although after the conclusion, in EFSA's Internet-page it is noted that BPA has been known since the 1930s to be able to mimic the female sex hormone, oestrogen and that its effects on fertility, reproduction and the endocrine system have been subject to much scientific debate.

EFSA's role in the EU food safety system is to carry out scientific risk assessment, which will inform the decision-making of EU risk manager in the European Commission, European Parliament and Member States. In addition to risk assessment, risk managers take into account other factors when making risk management decisions. (EFSA 2015c.) Because this risk assessment of EFSA is rather new, the following regulations and actions of EU and member states will show how the health risks of BPA will be noticed in the future.

2.1.2 Defining and testing biodegradation

Degradation of organic chemicals in the environment influences exposure and it is a key parameter for estimating the risk of long-term adverse effects on the biota. The degradation rates or half-lives can be determined laboratory-based degradation tests. Information on the degradability may be used for hazard assessment, such as classification and labelling, or risk assessment and persistency assessments. (ECHA 2014, 170.)

Degradation processes can be abiotic (hydrolysis, oxidation and photolysis) or biotic, which is commonly known as biodegradation. Biodegradation can be aerobic or anaerobic, depending on the presence of oxygen. Biodegradation is often defined as primary or ultimate. Primary biodegradation describes the initial transformation of a chemical by micro-organisms to another organic chemical, a transformation product or a metabolite. Ultimate biodegradation describes the multistep degradation process leading to inorganic end-products and biomass. In risk assessment, both ready biodegradability and inherent biodegradability tests can be used. The latter offers a higher chance of detecting biodegradation and therefore if an inherent test is negative, it could indicate persistency. (ECHA 2014, 170.)

OECD (Organisation for Economic Co-operation and Development) guideline defines six methods used for ready biodegradability assessment in an aerobic aqueous medium (OECD 2015). The following pass levels of biodegradation may be regarded as evidence of ready biodegradability: 70% DOC removal (TG 301A and FG 301E), 60% theoretical carbon dioxide (ThCO2 and TG 301B), 60% theoretical oxygen demand (ThOD, TG 301 C, TG 301D and TG 301F). These pass levels have to be reached in a 10-day window within a 28-day period of the test, apart from some exceptions. For example the test can be terminated before 28 days if the pass level is obtained. When a lower degradation is reached, the results need to be interpreted with caution, because of the possibility that the test was too short and a 28-day duration could have lead to degradation. However, these methods are not applicable for all substances and OECD is reviewing new guidelines considering for example wastewater. (ECHA 2014, 183, 193, 208.)

2.1.3 BPA degradation and the effects on the environment

European Union Risk Assessment Report (EU-RAR) on BPA notes that BPA released to the atmosphere is likely to be degraded by reaction with hydroxyl radicals of the atmosphere (half-life of 0.2 days). The physical

and chemical properties of BPA suggest that hydrolysis and photolysis are likely to be negligible. (EC 2003a, 42.)

However, degradation in an aquatic environment is not unambiguous. A number of biodegradation studies were summarized in EU-RAR 2003. EU-RAR 2010 offers an updated version of the risk assessment to be read together with the previous version. In the OECD's 301F manometric respirometry test, BPA met the criteria for ready biodegradability. However, in the OECD's 301D closed bottle test and OECD's 301B modified Sturm test no biodegradation was observed. In a modified SCAS procedure, BPA met the criteria for inherently biodegradable substances, although this test cannot give any indication of the potential for BPA to undergo ready biodegradation. (EC 2003a; 2010, 22.)

According to EU-RAR 2010, the measured levels of BPA before and after wastewater treatment at a chemical plant and major users of BPA suggest a high level of removal. In the report, it was not confirmed if this was happening via adsorption to sludge or biodegradation, although biodegradation was considered most likely to be the major removal mechanism. Based on biodegradation studies, BPA has been considered readily biodegradable, possibly with a short period of adaptation. The default rate constant for biodegradation in a wastewater treatment plant is stated to be $k=1h^{-1}$ for a readily biodegradable substance meeting the 10-day window. The resulting fate in a wastewater treatment plant was estimated by EUSES (EU System for the Evaluation of Substances) as 12% to water and 6.2% to sludge, with 81.9% degraded and a negligible fraction to air. (EC 2010, 22.)

A number of studies on the degradation of BPA in natural waters, were also summarized in EU-RAR 2003. According to the report, removal appears to be rapid once the waters have become acclimatised to BPA. The reported lag-phases before degradation have been 3-8 days. After the lag phase, removal was rapid with 50% removal in 1-2 days and 100% removal in 2 to 17 days. Based on these data, BPA appears to be classed as readily biodegradable meeting the 10-day test window. (EC 2010, 22.)

As one specific example, Kang and Kondo (2002a) found that spiked river water samples were rapidly biodegraded under aerobic conditions (>90%), while under anaerobic conditions the decrease of BPA concentration was hardly found (<10%) over 10 days. It was also found that BPA biodegradation by micro-organisms is influenced by temperature and microbe counts. Two strains that have high BPA biodegradability (90%) were undefined as a *Pseudonomas* sp. and a *P.putida* strain (Kang & Kongo 2002b.) According to their later study, in the case of seawater there was no relationship between the BPA degradation and the change of bacterial counts. It was found out that BPA can continue longer time with no degradation in seawater than in river water. (Kang & Kongo 2005.) Recent studies confirm that BPA is not biodegradable during the anaerobic digestion (Limam et al. 2013; Kim & Cunnigham 2014). The decrease of the BPA concentration appears to be due to its adsorption on solid waste and not to biodegradation. This was suggested to be a reason why BPA is found at very high concentration levels in sanitary landfill leachates and in anaerobic digestion digestates. (Limam et al. 2013.)

In previous EU-RAR 2003, the effects on aquatic environment were divided into toxicity test results and endocrine disrupting effects. In EU-RAR 2010, this section has been completely reformatted and updated. Given the rapid biodegradability of BPA in aquatic systems, studies that do not involve the confirmation of exposure concentrations have limited usefulness for PNEC derivation, especially over longer durations. Nevertheless, it is noted that such studies may still be considered qualitatively. (EC 2010, 55.) According to a number of acute toxicity studies for fish and saltwater fish, invertebrates and algae, the $L(E)C_{50}$ values are typically in the range 1-10 mg/L. Based on the studies, EU-RAR 2010 defined **$PNEC_{water}$ as 1.5 µg/L** and **$PNEC_{marine\ water}$ as 0.15 µg/L .** (EC 2010, 127.) For comparison, the PNEC values for BPA are in Canada 0.175 µg/L and in Japan 1.6 µg/L**.** It should be noted that countries use different approaches for generating PNECs and the values may differ even when based on the same studies. (U.S.EPA 2010, 9.)

One of the overall conclusions of the EU-RAR 2003 was that there is a need for limiting the risks on the environment (water and sediment compartments). The EU-RAR 2010 concluded that there is still need for further information and testing considering the freshwater and marine aquatic compartments including sediment. PNEC values may also be revised based on future studies. BPA was evaluated not considerably bio accumulative, so there was no risk considered for the terrestrial compartment or birds. (EC 2003a, 7; 2010, 124-128.) If BPA reaches the soil compartment, it is not expected to be stable, mobile or bioavailable - a dissipation half-life of less than 3 days has been estimated (Fent et al. 2003).

2.1.4 Biodegradation as a BPA removal technique from wastewater

Biodegradation has been proved to be an advanced technique to remove various pollutants from the environment. According to the studies of BPA degradation, plenty of BPA-degrading bacteria have been isolated and used to treat BPA in wastewater treatment. (Zhang et al. 2013.)

Bacterial strains capable of growing on BPA included gram-negative strains *Sphingomonas* sp., *Pdeudomonas* sp., *Achromobacter* sp., *Novosphingobium* sp., *Nitrosomonas* sp., *Klebsiella* sp. and *Cupriavidus* sp. and gram-positive strains *Streptomyces* sp. and *Bacillus* sp. Although there are many degrading bacteria in the environment, their ability to degrade BPA is strongly different depending on the strain specificity. However, the bacteria with high BPA biodegradability are limited, one being *Streptomyces* sp. [Kang et al. 2004]. (Zhang et al. 2013.) Also two rhizobacteria (*Sphingobium Fuliginis* TIK1 and *Sphingobium* sp. IT4) have been found useful for sustainable treatment of polluted waters containing various phenolic EDCs, including BPA (Toyama 2013).

Thus the biodegradability of BPA is one main part of this study, biodegradation as a treatment technique is not the main issue, and recent research is not thoroughly covered here.

2.1.5 Legislation and restrictions in the EU

The use of BPA is restricted in food contact materials in the European Union (Regulation 10/2011/EU), and banned in the manufacture of infant feeding bottles (Directive 2011/8/EU). Prior to EU regulation, some countries in Europe have had their own national regulation concerning the BPA in food contact materials or baby pacifiers. In addition, the usage in thermal paper has been restricted in Japan, Taiwan and Connecticut in USA. BPA was also included in the list of proposed priority substances of EU water policy directive amendment (2013/39/EU), but it was not confirmed. BPA is neither included in the Authorisation List nor in the Candidate List of Substances of Very High Concern for Authorisation of REACH, Appendix 14 (19907/2006/EU). (Ministry of Environment in Finland 2014, 19.)

France has made a proposal to change Appendix 17 of REACH and restrict the use of BPA in thermal paper in order to protect pregnant women, workers and consumers. The public consultation of the proposal was until the end of the year 2014. Restrictions would decrease remarkable the use of BPA, but there is also a risk of even greater health risks of the substitutes, such as bisphenol-S (BPS). (ANSES 2014; Ministry of Environment in Finland 2014, 20.)

In Finland, various restrictions in environmental permits of production plants can be set, considering for example wastewater treatment or replacing the BPA used in production. Although replacing has been considered problematic, because the substitutes can also have risks and there is not yet enough research and information about the risks. The Ministry of Environment has proposed environmental quality norms of BPA for the surface waters, which are based on the risk assessment reports of the EU (EC 2003 & EC 2010). This proposal is still a draft version. The mean concentration of the year (AA-EQS) would be in the inland surface waters 1.5 µg/L and elsewhere (seawater) 0.15 µg/L. It should be noted that the risk assessment of EU did not take into account the endocrine disruptive effects of the BPA, which have been detected remarkable lower

concentrations. Because there are no criteria for endocrine disruptive effects, it has not been possible to set norms taking into account these effects. (Ministry of Environment in Finland 2014, 21.)

2.2 Advanced oxidation processes

According to previous findings, BPA contaminates surface waters even at low concentrations. BPA cannot be entirely removed from water using conventional treatments and also such treatments can lead to by-products with even higher endocrine disrupting actor. Advanced oxidation processes (AOPs), especially the photo-Fenton process can be applied to remove BPA from water resources or industrial wastewater. (Oller et al. 2011; Pérez-Moya et al. 2014.)

Wastewater treatment consists often of three phases. In primary treatment, the physiochemical processes are used in order to reduce the concentration of oil, grease and various solids. Next, there is the secondary treatment, where dissolved organic matter will be biologically transformed for easy removal. Finally, tertiary treatment is for removing the specific contaminants. The need and ways of tertiary treatment depend on the end use of the water and pollutant loads. The AOPs are an alternative treatment when the water is contaminated with toxic and harmful micro-organisms difficult to eliminate biodegradability. AOPs can be used in an urban wastewater treatment plant in order to decrease final toxicity. AOPs can also increase biodegradability of industrial wastewater before conventional biological process as well as nontoxic forms of metals can be removed or converted (Figure 1). (Pignatello 2006; Rizzo 2011.)

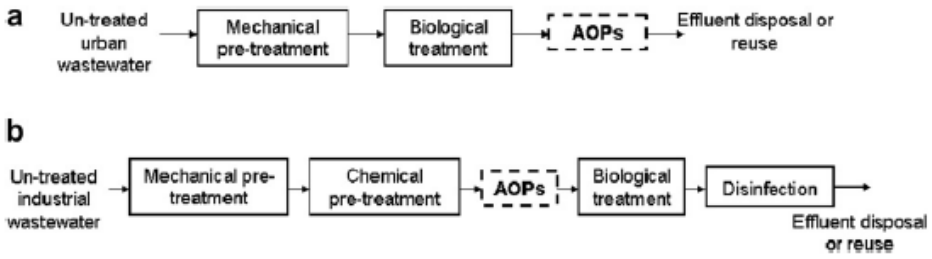


Figure 1. Application of AOPs to wastewater treatment: advanced treatment in urban wastewater treatment plant (a) and pre-oxidation to increase biodegradability before conventional biological process in industrial wastewater treatment plants (b). (Rizzo 2011.)

The AOPs can be broadly defined as oxidation methods that involve the formation of hydroxyl radicals (·OH) having higher oxidation potential than other traditional oxidants (ozone, hydrogen peroxide, chlorine dioxide or chlorine). These radicals are able to oxidize and degrade organic compounds by hydrogen abstraction or electrophilic addition to double bonds obtaining in some cases a complete mineralization. The advantages of this type of treatment versus other methods are:

- Pollutants do not only change phase, but they are transformed chemically
- In many cases complete mineralization of the pollutant is achieved
- The sludge that requires subsequent treatment process is not generated
- AOPs are useful for the treatment of refractory pollutants that resist other methods
- AOPs are also successful to treat pollutants in low concentrations
- AOPs consume less energy than other methods, such as incineration
- Pollutants are transformed into products that can be treated by more economical and more efficient methods
- There is no harmful effect on the health caused by residual disinfectants and oxidants generated by these processes

The main disadvantage of these treatments is the high operational cost compared to other conventional biological treatments. Chemical oxidation consumes energy and chemical reagents, which also increases treatment time. (Pignatello 2006; Rizzo 2011; Navarro 2013.)

AOPs can be classified into photochemical and non-photochemical processes. As Navarro (2013, 22) noted, photochemical degradation reactions are dependent on the characteristics of the source of light radiation [Wei 1991] and in many kinetic studies it has been verified that the rate of degradation increases with the increase of light intensity with a nonlinear relationship [Davis 1989].

In recent years, many AOPs have been studied to degrade BPA in wastewater. Some of the reagents used together with H_2O_2 have been TiO_2, Fe(II) and EDDS (Ethylenediamine-*N*,*N'*-disuccinic acid) complex. (Watanabe et. al. 200; Katsumata et al. 2004; Huang et al. 2013.) Different catalysts have also been investigated, such as ZnO, ZrO, CdS, MoS_2, Fe_2O_3, WO_3 (Rizzo 2011). Jiang et al. (2013) studied use of SO_4^- for degradation and mineralization of BPA. They concluded that sustainable supply of Fe^{2+} with slow flow rate could make the best use of generated SO_4^- . (Jiang et al. 2013). However, to continue Navarro's (2013) research, the photo-Fenton reaction is the main interest in this study.

2.3 Fenton, Fenton-like and photo-Fenton reactions

2.3.1 Reaction mechanisms

In the Fenton reaction, the interaction of H_2O_2 and iron salt (II) products hydroxyl radicals. The reaction is mainly based on series of chain reactions between Fe(II)/Fe(III) species and H_2O_2, especially at acidic pH value. The generally accepted mechanism of Fenton and Fenton-like reaction is described by Haber & Weiss (1934).

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + \cdot OH + OH^- \quad \text{(Fenton: fast)} \quad (2)$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + HO_2 \cdot + H^+ \quad \text{(Fenton-like: slow)} \quad (3)$$

The overall mechanism is catalytic as long as hydrogen peroxide is in excess compared to the amount of added iron. When the light is added to Fenton process, there are more active hydroxyl radicals produced faster and the degradation rate increases considerably (photo-Fenton).

$$Fe^{3+} + H_2O_2 \rightarrow FeOH^{2+} + H^+ \quad (4)$$

$$FeOH^{2+} \rightarrow^{light} Fe^{2+} + \cdot OH \quad (5)$$

In the dark conditions the reducing Fe^{3+} to Fe^{2+} is caused by Fenton-like process, when the limiting reagent of the reaction is available Fe^{2+}. In

photo-Fenton process, the reduction of ferrous iron is generated faster due to the light, and the limiting reagent is hydrogen peroxide. Photo-Fenton can be performed using low-energy photons located in the visible spectrum, which makes the process as low cost alternative. (Navarro 2013, 23.)

Navarro (2013, 23) noted that recent studies suggest the presence of more reactive intermediates and additional reactions, and the mechanism proposed by Haber and Weiss [1934] have been questioned for example by Goldstein & al. [1993] and Bossman & al. [1998]. Competitive reactions between H_2O_2 and hydroxyl radicals occur, which makes it challenging to find the right amount of Fenton reagents.

2.3.2 Variables influencing the photo-Fenton process applied for BPA

As a complex process, there are many variables effecting on the performance of mineralization - the most important being the concentrations of reagents, pH and the temperature.

Katsumata et al. (2004) concluded that comparing the results of the photo-Fenton reaction to TiO_2 photocatalyzed reaction (common AOP reagent) under UV irradiation, the first one is the possible method for the degradation of BPA from the viewpoints of degradation, time and cost. The amount of catalysis was ca. 90 times greater and time of degradation 2 hours under the experimental conditions of Watanabe's study of TiO_2 [Watanabe et. al.2003]. Katsumata et al. concluded that BPA was degraded in 9 minutes in the optimal conditions of their study. (Katsumata et al. 2004, 301.)

Usually, the stoichiometric ratio of [H_2O_2 /Fe(II)] could be appropriate from a technical point of view, but economic and environmental optimal concentrations depend on the effluent to be treated. Katsumata et al. (2004, 301) noted that usually H_2O_2/Fe(II) ratios from 10:1 to 40:1 are recommended optimal for the Fenton process. Furthermore, based on the

results of his study with BPA, the optimal ratios for the photo-Fenton were from 9:0.25:1 to 9:0.9:1 (H_2O_2/Fe(II)/BPA). Navarro (2013, 9) concluded that when treating BPA, the optimal ratio based on her results was around 6/3/1 (5,36/0,37/1,00; 5,36/0,25/1,00 and 6,70/0,33/1,00).

It has been discovered that the photo-Fenton usually is more efficient in acidic solutions. The degradation rate of the BPA is also highly dependent on pH. Katsumata et al. (2004, 299) concluded that optimal pH in their experiment was obtained as pH 4,0, although generally the optimal pH is around 3. For example Huang (2013) have also used BPA as model pollutant when studying conditions close to neutral pH, which is more natural for aquatic solutions. In his study in the Fenton and the photo-Fenton reaction Fe(III)-EDDS complex (Ethylenediamine-*N*,*N'*-disuccinic acid) was used in order to make the reaction work in neutral pH. However, this study is based on Navarro's (2013, 71) conclusion that implying the photo-Fenton on BPA, the optimal reaction rate and the maximum conversion occurs when the pH is close to 3.

According to Navarro (2013, 25), the rate of oxidation increases when the temperature increases. The recommended working range is 25-45ºC. At higher than 50ºC, the H_2O_2 decomposes into oxygen and water.

2.4 Indicators of the quality of the water

When using a combination of AOP and a biological process for treating recalcitrant contaminants, biodegradability assessment is required during the AOP treatment. There are several techniques used in this purpose, such as

- Analysis of general parameters, biological oxygen demand (BOD_x), chemical oxygen demand (COD) and dissolved organic carbon (DOC)
- Calculation of the BOD5/COD ratio or the average oxidation state (AOS)
- Long activated-sludge biodegradability assays, such as Zahn-Wellens test, which takes 28 days

- Oxygen uptake rate by respirometric measurements (about 20 minutes)

Other techniques have also been developed, but in general the majority of studies in this field employ conventional bioassays, such as biological oxygen demand (BODx/COD rate), to determine enhancement of the biodegradation rate after pre-treatment by AOP. (Oller 2011.)

2.4.1 Biochemical Oxygen Demand (BOD)

The biochemical oxygen demand (BOD) of water is an expression for the amount of oxygen consumed by the decomposition of organic matter in a biochemical process. BOD is used in evaluating the quality of wastewater, industrial effluents and surface water. Practically the reaction can often be considered complete in 20 days. However, it is too long in most cases and it has been found by experience that a reasonable large percentage (70-80%) of the total BOD is exerted in 5 days. The exact percentage depends upon the character of the "seed" and the nature of the organic matter and can be determined only by experiment. (Sawyer & all. 2003, 604.) Therefore often BOD is analyzed using the value of 5 days incubation (BOD_5), although in some countries, such as Finland, the BOD_7 is commonly used (Finnish Environment Institute 2014). Depending on the measurement, other durations can also be used (BOD_n).

BOD is traditionally measured using the unit mg/L, when biodegradability has been calculated by measuring the consumed oxygen in the beginning and the end of the experiment. Oxygen concentration can be analyzed by using the Winkler titration or oxygen electrode (SFS-EN 25813, SFS-EN 25814). This method may lead into an inaccuracy in the results, because there is a technical limit for oxygen concentration (9 mg/L) and too high concentration may influent on results. Making dilutions also may need several replicates. (Prokkola & Kuokkanen 2011.)

Manometric respirometric method solves many problems traditional methods have. This method is based on a closed system, where changes

in pressure are measured. Biodegrading consumes oxygen and releases CO_2, which reduces the pressure. The equipment calculates the BOD value using the formula of ideal gas law. If the biodegradability of a certain compound is measured, the concentration of carbon has to be known in order to calculate the biodegradability rate. For better accuracy also the concentration of hydrogen should be known. (Prokkola & Kuokkanen 2011.)

The BOD tests are designed to measure the oxygen requirements by the oxidation of organic matter present in samples. Therefore, it is important that no organic matter from outside sources is present. Since it is impossible to exclude extraneous organic matter in the BOD test, blank samples are required in determination. (Sawyer, McCarty & Parkin 2003, 627.)

The Dilution Water. Through long experience it has been realized that synthetic dilution water prepared from distilled or demineralised water is best for BOD testing, because most of the variables can be kept under control. The pH may range from 6.5 to 8.5, but it is customary to buffer it at about pH 7,0. The proper osmotic conditions are maintained by the potassium and sodium phosphates added to provide buffering capacity. In addition, calcium and magnesium salts are added which contribute to the total salt content. These salts also provide the micro-organisms these elements that are needed in growth and metabolism. Ferric chloride, magnesium sulphate and ammonium chloride supply the requirements for iron, sulphur and nitrogen. The phosphate provides phosphorus that may be needed. The nitrogen should be eliminated in cases where nitrogenous oxygen demand is being measured. The dilution water should always be "seeded" with wastewater or other material to ensure a uniform population of organisms. (Sawyer et al. 2003, 611-12.)

Dissolved oxygen (DO) measurement was needed as part of BOD analysis for the calculation of the seed control factor. Dissolved oxygen refers to the level of free, non compound oxygen present in water. The bonded oxygen molecule in water (H_2O) is in a compound and does not

count in measurements. Dissolved oxygen can be presented as mg O_2/L, but often dissolved oxygen saturation is used. 100% air saturation means that the water is holding as many dissolved oxygen molecules as it can in equilibrium. The two bodies of water can have the same air saturation, but actual amount of dissolved oxygen varies depending on temperature, pressure and salinity. (Sawyer et al. 2003.)

2.4.2 Chemical Oxygen Demand (COD)

The chemical oxygen demand (COD) test is widely used measuring the organic strength of domestic and industrial wastewaters. It is based on the fact that nearly all organic compounds can be oxidized by the action of strong oxidizing agents under acid conditions. During the determination of COD, organic matter is converted to carbon dioxide and water regardless of the biological qualities of the substance. COD values can be much greater than BOD values when significant amount of biologically resistant organic matter is present, such as glucose or lignin. The major advantage of the COD test is the short time required. The determination can be made in 3 hours instead of BOD, which takes 5 days. For this reason it can be used as substitute for BOD test, if reliable correlation between COD and BOD is evaluated by sufficient experience. (Sawyer et al. 2003, 625.)

The chemical oxygen demand is defined as the amount of oxidant that reacts with the sample and the result is expressed in mg/L (O_2). This represents the amount of oxygen equivalent to oxidizing chemical used in process. Potassium permanganate (COD_{Mn}), ceric sulphate, potassium iodate and potassium dichromate (COD_{Cr}) can be used as oxidizing agents, the latter being the most practical with wastewater. (Sawyer et al. 2003, 626.)

The COD test is precise and accurate for samples with COD of 50 mg/L or greater. For more dilute samples, the analysis requires extra carefulness and good analytical techniques in order to obtain accurate results. (Sawyer et al. 2003, 628.) In this study, the COD test was not implemented,

because the presence of Fenton reagents affects the COD test. The theoretical COD value based on the TOC was used instead.

2.4.3 Total Organic Carbon (TOC)

Measuring the amount of organic matter can be directly analyzing the total organic carbon (TOC) or indirectly reducing the capacity of the existing carbon in the sample by determining the BOD and COD. TOC is faster and more accurate method. In measuring BOD and COD, organic and inorganic nitrogen and hydrogen can contribute to the oxygen demand due to e.g. nitrification or some inorganic ions can cause interferences. (Sawyer et al. 2003, 629.) By analysing BOD and TOC, theoretical COD can be calculated.

Monitoring TOC during the photo-Fenton reaction, it is possible to determine the evolution of the mineralization of BPA. When all the organic substances have mineralized, the TOC value gets close to the zero.

2.4.4 Relations of BOD, COD and TOC

COD data can also often be interpreted in terms of BOD values after sufficient experience has been accumulated to establish a reliable correlation between COD and BOD. (Sawyer et al. 2003, 625.) BOD/COD ratio is also often used when evaluating the quality of the water and the biodegradability of the contaminants in the water (Table 2).

By theoretical calculation of the parameters (BOD, COD and TOC) it is possible to estimate theoretical values of others. The relationship between TOC and COD is based on the stoichiometry, so the theoretical COD can be calculated from TOC [Metcalf 2003]. (Navarro 2013, 28.)

$$TOC_{Theor.} = \frac{[substance(mg/L)] \times 12 \times number\ of\ C\ in\ substance}{M\ substance} \quad (6)$$

$$= \frac{30 \times 10^{-3} g}{l} \times \frac{1\ mol}{228.29\ g} \times \frac{15\ mol\ C}{1\ mol\ C_{15}H_{16}O_2} \times \frac{12\ g}{1\ mol\ C} \times \frac{1000\ mg}{0.98\ g}$$

$$= 24.14\ mg/L$$

$$\frac{COD}{TOC} = \frac{32}{12} = 2.667 \quad (7)$$

$$COD_{Theor.} = \frac{[substance(mg/L)] \times 32 \times number\ of\ C\ in\ substance}{M\ substance} \quad (8)$$

$$BOD_{5_{Theoretical}} = (\%) \times COD \quad (9)$$

Table 2. Biodegradability and BOD/COD ratio [Garciá- Montaño 2006]. (Navarro 2013, 29.)

BOD /COD	**Biodegradability of the substance**
> 0.6	Totally biodegradable
0.41 - 0.59	Partially biodegradable
< 0.4	Not biodegradable

2.5 Toxicity

2.5.1 Toxicity of BPA

In Chapter 2.1.1, the results of EFSA's risk assessment on BPA, and their scientific opinion in 2015 were presented. In the EFSA's article (2015c), it is stated that the limited number of large scale toxicity studies complying with standard/OECD test guidelines have consistently indicated that the oral toxicity of BPA is low. Still, there have been many more small-scale research studies that have reported adverse effects of BPA at levels below the previous NOAEL of 5 mg/kg bw/day, which was the point of departure for the derivation of the previous TDI.

The TDI defined in the EU Risk Assessment reports (EU-RAR) 2003 and 2010 was based on the experimental studies in rats, mice and dogs. In 2010, EFSA further confirmed the validity of NOAEL value with systemic effects identified in the multi-generation studies in rats and mice. Likely, adverse effects in animals on kidney and mammary gland underwent benchmark dose response modelling ($BMDL_{10}$). Using data on toxicokinetics, this $BMDL_{10}$ was converted to a human equivalent dose (HED). Then EFSA's CEF panel applied a total uncertainty factor of 150 to establish a new t-TDI value of 4 µg/kg bw/day (previously 50 µg/kg bw/day). (EFSA 2015c, part II, 4, 67, 76.)

According to the EU-RAR, for the environment BPA has acute L/EC_{50}s in the range 1-15.5 mg/L, it is considered biodegradable and not bioaccumulative (EC 2003b, 12). It is concluded that BPA is not PBT (persistent, bioaccumulative and toxic) or vPvB (very persistent and very bioaccumulative) substance, but it meets the toxicity criterion (EC 2010, 131).

Navarro (2013) has collected eleven possible toxic by-products into the table (Appendix 15). Katsumata et al. (2004) determined six of these intermediate products during the photo-Fenton experiments with BPA and the others were found by Poershemann et al. [2010] and Rodrigues, et al [2010]. (Navarro 2013, 75.) The by-products are not necessarily toxic or last long, but their existence justifies the need for the further study of toxicity of the substances and the evolution of toxicity during the photo-Fenton reaction.

2.5.2 Toxicity testing methods

Rizzo (2011) has summarized the most often used toxicity testing methods together with AOPs. The test organisms can be grouped into micro-organisms, plants and algae, invertebrates and fish. The most used invertebrate is *Daphnia Magna*, but there are other tests using also *Artemia salina* and sea urchin. Because of the high sensitivity of some invertebrates to high polluted aqueous matrices, such as industrial

wastewater, these organisms may not be useful to characterize toxicity. Plant based bioassays have often been used to evaluate the toxicity of organic and inorganic contaminants, contaminated soils, solid waste and sludge. Methods based on the inhibition of algal growth have also been developed. Preferred species in fish bioassays are rainbow trout (*Oncorhynchus mykiss*) and bluegill sunfish (*Lepomis macrochirus*). (Rizzo 2011.)

Microbial bioassays include a wide variety of techniques available for toxicity screening purposes. They can be based on 1) the capacity of micro-organisms to transform carbon, sulphur or nitrogen, 2) enzymatic activity, 3) growth, mortality or photosynthesis, 4) glucose uptake activity, 5) oxygen consumption or 6) luminescence output. Luminescent micro-organisms have been used in the production of many toxicity tests. Toxicity testing with marine bacterium *V. fischeri* has been routinely used as fast, practical, reliable and sensitive test protocol for industrial pollutants. (Rizzo 2011.)

Chen et al. (2006) studied the endocrine effects during BPA degradation. Changes in estrogenic activity were evaluated using both in vitro yeast estrogen screen and in vivo vitellogenin assays with Japanese medaka fish (*Oryzias latipes*). In another study the acute toxicity of untreated and photo-Fenton-like treated BPA samples was measured with a BioTox^{TM} test kit (Aboatox Oy, Finland; *V. fischeri* code 1234-500). It is a commercial bioassay based on the inhibition of bioluminescence emitted by the marine bacteria *V*. *fisheri* in accordance with the ISO 11348-3:2007 protocol. (Molkenthin et al. 2013.) Kim et al. (2000) studied several EDCs including BPA using five freeze-dried recombinant bioluminescent bacteria. They found out that these bacteria could be used to analyse toxic effects of EDCs within practical dosage ranges.

Rizzo (2011) reviewed critically the methods used in toxicity and biodegradability evaluation, particularly when AOPs are investigated in industrial wastewater pre-treatment. According to Rizzo, some authors use toxicity tests to infer the behaviour of treated wastewater in terms of

biodegradability in relation to a subsequent biological process as well as to set up an optimum operating condition of the investigated AOPs. In his opinion, this approach is incorrect, because depending on the organism used in toxicity tests, the results may either underestimate or overestimate the effect of AOPs on the biodegradability of wastewater. Different organisms used for toxicity test can give different results in terms of toxicity. Rizzo pointed out that it is possible to make a mistake, if one is looking for a relationship between toxicity and biodegradability, when the investigated aqueous matrix is toxic to organisms used in bioassay, but not toxic to bacteria which promote biodegradation process. (Rizzo 2011.)

As a conclusion, Rizzo noted that sometimes acute toxicity tests may not be the most suitable to evaluate the ecotoxicological hazard of micropollutants because of the low concentrations. Toxicity tests may also not be suitable when evaluating the effect of AOPs on biodegradability, but they could be used just as a screening test before to use more suitable biodegradability tests (e.g. activated sludge bioassays and respirometry). (Rizzo 2011.)

3 ANALYTHICAL METHODS AND TECHNIQUES

3.1 TOC Analyzer

Equipment:
TOC analyzer,
Shimadzu TOC-$V_{CSH/CSN}$

TOC measurement range
between 0.03 and 1000 mg C / l

Picture 1. TOC analyzer Shimadzu

The operation of the TOC analyzer is based on an oxidation catalytic combustion and non-dispersive infrared detection (NDIR). The NDIR detects the CO_2 concentration and the detector generates a signal peak, which area is proportional to the concentration of total carbon (TC) and inorganic carbon (IC). The measure of TOC is calculated by the difference between TC and IC, whose units are mg C/L or ppm C. (Lovibond 2011.)

Determination of TC is performed in 680ºC catalytic combustion, which oxidizes the organic and inorganic carbon to CO_2. The IC comes mainly from dissolved CO_2, carbonates and bicarbonates. The system automatically adds the acid (HCl) to the samples when needed. The high specificity of the detection technique is due to the fact that infrared spectra are characteristic of each substance, presenting spectra of gaseous samples sharply. (Lovibond 2011.)

Before measuring TC and IC, the suitable calibration curves were chosen from the equipment. Measuring TC it was chosen to first use the curve 1 (concentration of the contaminant 5-50 mg/L) and secondly the curve 0 (0-20 mg/L). After first measurement the device then automatically chooses the more accurate curve. Minimum injections were chosen as 2 and

maximum as 3, when standard deviation (SD) and variation (CV%) were automatically given (Table 3). The equipment is then set to measure minimum injections needed, but if SD and CV% limits were exceeded, the third measure will be taken. The mean value is given as the result and unreliable results are excluded. Reliability of the results can be evaluated by SD and CV% values.

Table 3. Characteristics used when measuring TC and IC

	Calibration curves	Volume of inj. (µl)	Inj. min.	Inj. max	SD	CV %	Dilution factor	Acid ratio
TC	1; 0	50	2	3	0,1	2	1	0
IC	0	540	2	3	0,1	2	1	1,5

3.2 Spectrophotometer and the determination of hydrogen peroxide

3.2.1 Spectrophotometry and the equipment

Equipment:
Perkin Elmer UV/VIS Spectrometer, Lambda 2

Wavelength 190nm to 1100nm
Used: 450 nm
Reagents:
Metavanadat, NH_4VO_3
(purity 98,5%)

Picture 2. Perkin Elmer Spectrophotometer

Spectrophotometry is based on the fact that every chemical compound absorbs, transmits or reflects light over a certain range of wavelength. In spectrophotometry it is measured how much a chemical substance absorbs or transmits. Spectrophotometry is widely used for quantitative analysis in various areas. A spectrophotometer is an instrument that measures the amount of photons (the intensity of light) absorbed after the light passes through sample solution. (ChemWiki 2014.)

With the spectrophotometer it can also be determined the amount (concentration) of a known chemical substance by measuring the intensity of light detected. Depending on the range of wavelength of light source, the devices can be classified into two different types, UV-visible spectrophotometers (ultraviolet range 185-400nm and visible range 400-700nm) and IR spectrophotometers (infrared range 700-15 000nm). In visible spectrophotometry the absorption or the transmission of a certain substance can be determined by the observed colour. (Perkin Elmer 1991; ChemWiki 2014.)

Method 4 was selected from the spectrophotometer and the wavelength was set at 450 nm. First the absorbance of the zero-sample was measured and set as the zero for the measurements. Then the absorbance of the samples was read bearing in mind the proper handling the sample. The cuvette was first rinsed with the sample and then filled up. Always the same cuvette was used and the surface of the cuvette was kept clean and dry. The residual was placed into a waste container meant for metavanadat residual.

3.2.2 Determination of H_2O_2

To be able to determinate the H_20_2 of the moment the sample was taken, the reaction has to be stopped. The method used is based on the reaction of hydrogen peroxide with ammonium metavanadat in acid medium.

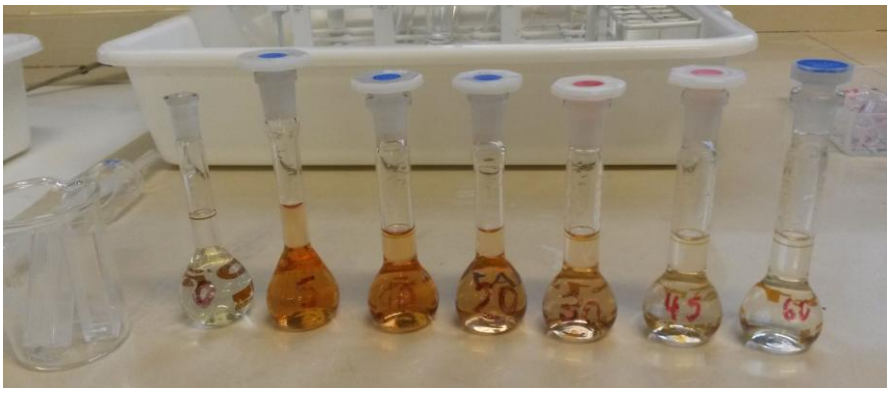
$$VO_3^- + 4H^+ + H_2O_2 \rightarrow VO_2^{+3} + 3H_2OVO_3^- + 4H^+ \quad (10)$$

$$VO_2^{+3} + 3H_2OVO_3^- + 4H^+ + H_2O_2 \rightarrow VO_2^{+3} + 3H_2O \quad (11)$$

Orange-red VO_2^{+3} has a maximum absorbance at 450 nm and can be tracked by spectrophotometry. To ensure that all H_2O_2 has reacted with metavanadat, the latter has to always be in excess. The final concentration of VO_2^{+3} is equal to the initial H_2O_2 by the stoichiometry of the reaction. Quantifying the concentration of cations it is possible to determine concentration of H_2O_2.

The determination of hydrogen peroxide was carried out according to instructions of the UPC laboratory, which can be found in Appendix 5. According to the instructions, each sample was measured into a 10 mL bottle consisting 1.1 mL of 0,062 M NH_4VO_3 and filled up with deionised water. Concentration of the solution is then 6,82 x 10^{-3} M, which is the possible maximum of H_2O_2. The method 4 and wavelength 450 nm were chosen from the equipment to measure the absorbance.

Each sample from the photo-Fenton experiment was 5 mL and samples were taken in 5, 10, 20, 30, 45 and 60 minutes (Picture 3). When the absorbance showed clearly that all H_2O_2 was consumed, the samples were not taken anymore while experiment still continued. There was available an excel sheet calculating the ratio of consumed H_2O_2, so it was possible to get the curve immediately the absorbance was read to make conclusions during the experiment. Calculation was based on the calibration curve presented later in Chapter 5.1.1.



Picture 3. 5ml samples taken during the experiment reacted with the ammonium metavanadat giving orange-red colour. Concentration of VO_2^{+3} was analyzed using spectrofotometer and amount of H_2O_2 calculated by stoichiometry.

3.3 BOD_5 and BOD_7

Biodegradation of BPA at different concentrations was analyzed in order to compare the results with the final samples of the experiments. The relevant sample size was also determined by having sample sizes of 360 mL and 428 mL when performing the first tests.

BPA solutions of different concentrations were stored in a refrigerator and analyzed as soon as possible. The final samples of the experiments were stored in a freezer and melted in a refrigerator and room temperature just before the BOD analysis. The measuring range was 0-400 mg/L and the final volume introduced into the bottle was 360 mL. The results of the preliminary BOD tests are presented in Chapter 4.3.2 and the BOD of the experiments in Chapter 5.2.2. Dissolved oxygen was measured from blank and seed control samples separately with handheld DO-meter (Appendix 7). Both BOD_5 and BOD_7 values were recorded and comparison of the results is presented in Chapter 5.4.1).

3.3.1 Equipment, solutions and the method

Picture 4. Lovibond OxiDirect BOD-system

Equipment:
Lovibond OxiDirect BOD-system

Max. 6 tests per experiment,
Measurement intervals (mg/L O_2):
0-40, 0-80, 0-200, 0-400, 0-800, 0-2000, 0-4000.

Other devices:
Frigotermostat
pH-meter
Magnetic stirrer
Handheld Oxi 330i/340i DO-meter, Crison

Table 4. Reagents and solutions for BOD (Appendix 2).

	Name	Formula	Concentration
a)	Phosphate buffer	NaH_2PO_4 x H_2O	23.81 g / 100 ml
		NH_4Cl	3.82 g / 100 ml
b)	Potassium hydroxide	KOH	6 M
c)	Calcium chlorate	$CaCl_2$	2.77 g / 100 ml
d)	Magnesium sulphate	$MgSO_4$ x 7 H_2O	10.1 g / 100 ml
e)	Ferrous chlorate	$FeCl_3$ x 6 H_2O	0.484 g / 100 ml
f)	Sodium hydroxide	NaOH	1 M
g)	Sulphuric acid	H_2SO_4	1 M
h)	Glutamic acid / Glucose 300 mg/L	$C_5H_9NO_4$ + $C_6H_{12}O_6$	4 g / L
i)	BOD water	a), c), d), e)	1+1+1+1 ml/L (pH 6,8-7,2)
j)	Seed Inoculum (*)		

*) PolySeed®: Prepared in accordance to the manufacturer's instructions. Content of the capsule (powder) is placed in 500 mL of BOD water prepared in accordance to Standard Methods. PolySeed® solution is aerated and stirred for one hour and then let settle down and decanted. In a clean 500 mL beaker the solution is stirred for the remainder of the test. NOTE: For best results, the solution should be used within 6 hours rehydration of the capsule. (InterLab 2012.)

The Biochemical Oxygen Demand (BOD) is a test that determines the Oxygen requirements concerning wastewater effluents and polluted water, for biological degradation. This test expresses the degree of contamination of wastewater per degradable organic matter by biological oxidation in 5 days (+20ºC, pH approximately 7). The equipment determines the consumed oxygen by reducing pressure inside closed system BOD using pressure sensors. Determination of the method is respirometric BOD. (Lovibond 2011.)

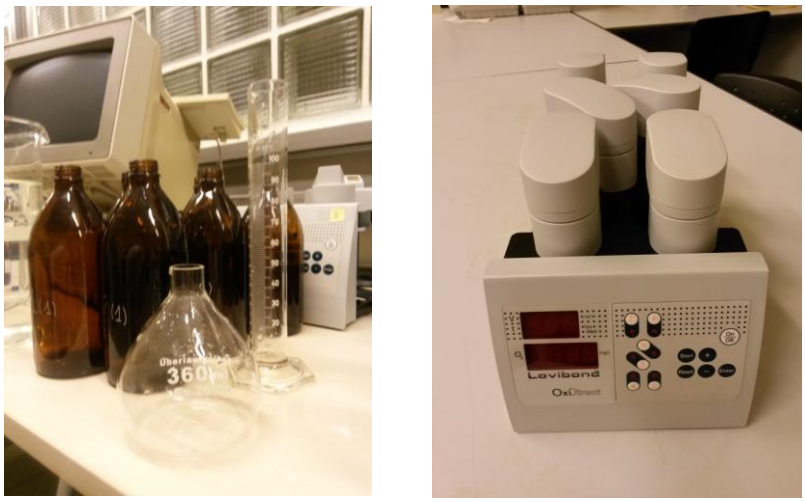
During the BOD determination of water, introduced bacteria consume oxygen dissolved in water. When this oxygen is consumed, it is replaced by oxygen, which is in the headspace of the bottle test. The carbon dioxide produced simultaneously by the same bacteria is chemically combined with a potassium hydroxide solution, which is placed in a small reservoir on the neck of the bottle. The decrease in pressure is measured by the sensors of the equipment. This pressure drop is directly proportional to oxygen consumption. (Lovibond 2011.)

As introduced bacteria, the PolySeed® capsules were used (InterLab 2012). To make sure that the seed works the way it should, seed controls were taken in accordance to Polyseed instructions (InterLab 2014). Seed factor was calculated and compared to manufacturer's instructions. Glutamic acid and glucose (GGA) solution was freshly made as an aliment. Concentration of 50 ppm was chosen (Chapter 4.5.3), where both compounds had equal share (25+25ppm). For practical reasons it was decided not to do GGA controls. There were only six bottles in one equipment and there would not have been space for all controls, so only the essential ones regarding the purpose of the study were carried out (Appendix 7).

3.3.2 Additional instructions of the UPC laboratory

BOD analyses were implemented using the manual of the manufacturer and the instructions of the laboratory of UPC Barcelona Collage of Engineering, which can be found in Appendix 2. Because the BOD was carried out in stabile laboratory conditions using deionised water instead of real wastewater, it was added certain minerals to the solutions of BPA and experiment samples in accordance to instructions (Chapter 2.4.1., Appendix 2). Each mineral solution was added into each sample and BOD water. The amount added was equal to 1 mL /1 litre of solution (Table 2 in Appendix 2). These solutions were made in accordance to the instructions. The pH of each sample solution was adjusted after adding minerals. Pictures 5-8 present the method in practice.

Pictures 5 & 6. The BOD samples were treated in accordance to instructions (Appendix 2), minerals were added and pH adjusted.



Pictures 7 & 8. Into each BOD bottle it was measured a proper amount of sample and ATH drops, PolySeed and GGA solutions were added. KOH was dropped into the lid, bottles closed carefully, measurement started and equipments placed into the frigothermostat to incubate in +20ºC for 5 days. (Appendix 2.)

3.3.3 Dissolved oxygen

The electrochemical method of measuring DO requires a cathode, anode, electrolyte solution and gas permeable membrane. The membrane is made from special material that permits oxygen to pass through. Oxygen is consumed by the cathode which creates a partial pressure across the membrane. Oxygen diffuses then into the electrolyte solution. (ThermoScientific, Eutech 2014.)

Dissolved oxygen was measured using Handheld Oxi 330i/340i DO-meter. Measurement takes few minutes and it was performed before closing the BOD bottles and again after BOD_7 value was read and the bottle opened again. Because BOD_7 values were read in addition to BOD_5 values, it was impossible to measure DO after 5 days. Based on the other results, the difference between BOD_5 and BOD_7 most likely was not remarkable, but it should be kept in mind when analysing the results.

3.4 Toxicity

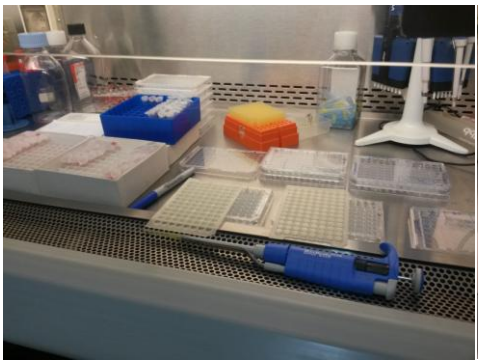
Equipment:	Bacteria:
Biochrom, EZ Read 400 Microplate reader and ADAP Software	*Escherichia coli* *Staphylococcus epidermidis*

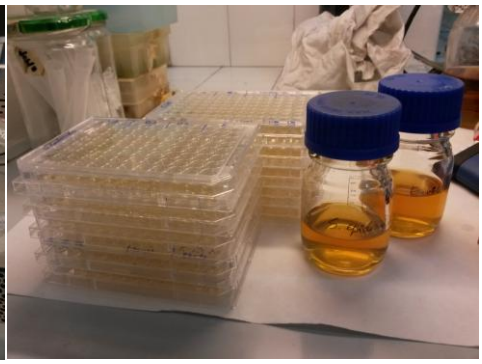
Toxicity analysis were performed using two different bacteria, gram negative *Escherichia coli* (DH5α) and gram positive *Staphylococcus epidermidis* (CECT 231). This method was chosen because it was the available method in UPC laboratory and *Escherichia coli* had been used also in the previous study of toxicity of 2-chlorophenol (Pérez-Moya et al. 2007).

Solutions for the analysis were prepared adding proper amount of bacteria solution into 100 mL of culture media. Concentration of each bacteria solution was first 1 million bacteria / 1 mL. 100 µL of this solution was pipetted into the samples. After first experiments it was realized that there was too much growth to be able to read the results and the concentration

was reduced to 1000 bacteria/mL and the analysis repeated again. Only the latter results were analysed.

As preparation, frozen toxicity samples were taken into the room temperature approximately 2 hours before analysis. There were three eppendorfs of each sample (3 x 1.5 mL), so for the analysis 100 µL sample was pipetted from each eppendorf to get three replicate samples (Pictures 9 & 10). When all the samples were pipetted, the rest were frozen again for the possible future use or replicates.

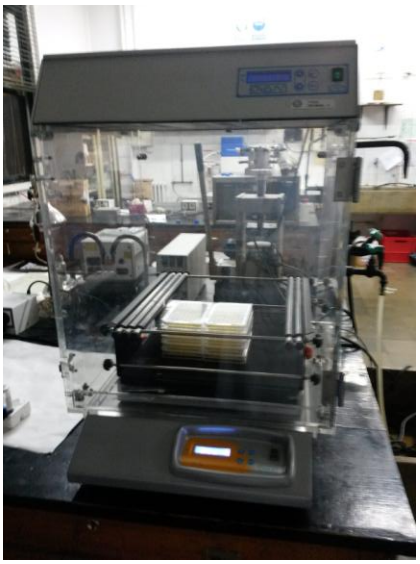




Pictures 9 & 10. Three replicates of each sample were pipetted in two separate plates. *E. coli* and *S. epidermidis* solutions (1000 bacteria/ml) were prepared and added to the plates.

Next 100 µL of the each bacteria solution was pipetted into separate sample plates, on top of the pipetted sample solutions (Picture 11). Six control samples were also made using 100 µL of sterilized water and 100 µL of bacteria solution. The purpose of the controls was to present the possible maximum growth of the bacteria. The way of presenting the results is the growth percentage in comparison to the maximum growth.

The samples were placed for 24 hours into a cabin (Picture 12), where the temperature was set at +37ºC and the sample plates were constantly shaken. The results were read with EZ Read 400 Microplate Reader using ADAP Software (Pictures 13 &14). Measurement filter was set at 650 nm and reference filter at 450 nm. The results were copied into Excel sheet for calculations and analysis.



Pictures 11 & 12. 100 µl of bacteria solution was added and the sample plates were incubated in 37ºC for 24 hours before reading.

Picture 13 & 14. Biochrom, EZ Read 400 Microplate reader.

4 EXPERIMENTAL METHODS AND PRELIMINARY RESULTS

4.1 The plan for implementing the study

Firstly, the solutions of different concentrations of BPA were needed in order to get comparative results of the TOC, BOD and toxicity. It was decided to make solutions having concentrations of 2.5; 5.0; 7.5; 10.0; 20.0 and 30.0 mg/L of BPA. For the experiments, the 30 mg/L of BPA-solution was chosen based on previous research (Navarro 2013). TOC, BOD and toxicity were analyzed from these six solutions.

These solutions were also used to determine if there was a difference in BOD results depending on the volume of the sample. BOD analyses were implemented using two volumes of the sample, 360 mL and 480 mL and the results were analysed in order to choose the best volume size for the experiment. The biodegradability of different concentrations of BPA was determined based on the results of BOD_5. BOD_5 and BOD_7 values were also compared to each other to see if there is significant difference on duration of the process.

Preliminary experiments were carried out in order to learn the technique and to find out the right duration of the experiment as well as effects and adjustment needs of pH and temperature. Based on the previous research and preliminary experiments, the final design of the experiment was determined (Chapter 4.5).

4.2 Method of the experiment

To be able to compare the results, the experiments were implemented the same way Navarro had . Equipment needed for the experiment were the following:

- A thermostatic cylindrical 500 mL Pyrex cell
- The lamp used was Ultra-Vitalux, Osram 300 W and 230 V

- A pH- meter Crison, thermometer included (most of the experiments were using separate thermometer), a crucible to protect the pH-sensor
- A water bath to regulate the temperature of the container
- A magnetic stirrer
- Automatic pipettes of 2-20µL, 20-200µL and 100-1000µL

Reagents needed were:

- H_2O_2, 33%, purity 98 %
- sample solution: BPA (30 mg/L), 500 mL/experiment
- $FeSO_4$ x 7 H_2O, Merck
- 1 M H_2SO_4
- 1 M NaOH

Picture 14. The set up for the experiments.

The conditions for the experiment were monitored and controlled if needed. Conditions were following:

Concentration of the contaminant (BPA): All the experiments are performed with the same concentration of the BPA, which is 30 mg/L. The same concentration had been used in previous research and to be able to compare the results, the same concentration was chosen

Concentration of the reagents: Both the concentration of hydrogen peroxide and the salt of iron (II) were variables of this study (Chapter 4.5).

pH: In Navarro's research the pH 3 was chosen based on her experiments and the research of Katsumata et al. 2004. Before each experiment, the pH was adjusted between 2.8-3.0 (often close to pH 2.9) and the values were recorded and monitored during the whole experiment. If the limits were exceeded, the pH was adjusted using 1M H_2SO_4 or 1 M NaOH.

Light: Light features are described above. The distance between the lamp and the surface of the solution in the beginning was 21 cm. The presence of the light was a variable in Navarro's preliminary study and in this study it was chosen to use only the photo-Fenton with light giving much greater degradation rate than without.

Temperature: The aim was to have 25 ± 0,5 ºC as in Navarro's study. During the first preliminary experiments it was realized that the warming effect of the light was remarkable, even 3,0 ºC. Since the water bath system was not directly able to cool the container, but only heat it and keep stable, it was tried to cool down and control the temperature by adding some ice in the water bath. This manual interfering could not be standardized and cooling down disturbed the process and affected the curves notably. So it was decided to let the process proceed without adjusting the temperature during the process, although temperature was monitored. It was assumed that heating during the 90 minute experiment would be nearly the same each time. Although after several experiments the conditions were warmer already in the beginning. The method used was to cool down the container in the beginning close to the 25ºC using ice and then just monitor the temperature. Effects of the temperature are discussed in Chapter 4.3.4.

Homogeneity: To ensure the homogeneity of the solution, the magnetic stirrer was fixed at 500 rpm.

Order of the sampling: To minimize the effects of human related methological error, the sampling was implemented in same order every

time. First were taken the toxicity samples, secondly the TOC sample and the last one was H_2O_2 sample. It usually took around 40-50 seconds to take all samples.

Duration: According to the DOE, in preliminary studies 60 minutes were used, but it was realized that 90 minutes is needed for the experiment (Chapter 4.5.2).

4.2.1 The coding of the experiments

It is essential to have a simple and informative coding for the experiments in order to present the results illustratively. The chosen coding is based on same systematic idea than previous studies in UPC, because then understanding and comparing results is easier for the reader. In the name of the experiment, the variables are separated by using underscore.

Firstly, there is the name of the contaminant (BPA) and the concentration (30 mg/L). After that the variables and other characteristics are listed in following order; hydrogen peroxide concentration, iron (Fe^{2+}) concentration (mg/L), presence of the light (all cases ON), number of repeated sample and adjustment of pH. In case of pH, it was either not adjusted (NA) or adjusted (A) in order to keep it at 2.9 $\pm$1. Adjusting was 0-3 times depending on the experiment. More specific information is presented in Appendix 9. Temperature was monitored and in some cases adjusted (Appendix 10), but not included in coding. More about effects of the temperature and pH is discussed in Chapter 4.3.4.

For example the second experiment, where H_2O_2 (161 mg/L) and Fe(II) (10 mg/L) were added and pH was adjusted during the experiment, was coded as BPA_30_161_10_ON_2_A. Because the concentration was the same (30 mg/L), and all experiments had the treatment with light (ON), often the short name is used when presenting the results. As a work name only the codes (A-K) were used. (Table 7.)

Table 7. The names and codes of all experiments. Because the concentration was the same and all experiments had the treatment with light, often the short name is used.

Code	Name of the experiment:	Short name:
A1	*BPA_30_40.25_5.0_ON_NA*	A1_40.25_5.0_NA
A2	BPA_30_40.25_5.0_ON_2_A	A2_40.25_5.0_A
B1	BPA_30_40.25_10.0_ON_NA	B1_40.25_10.0_NA
B2	BPA_30_40.25_10.0_ON_2_A	B2_40.25_10.0_A
B3	BPA_30_40.25_10.0_ON_3_A	B3_40.25_10.0_A
C1	BPA_30_161_5.0_ON_A	C1_161_5.0_A
C2	BPA_30_161_5.0_ON_2_NA	C2_161_5.0_NA
C3	BPA_30_161_5.0_ON_3_A	C3_161_5.0_A
D1	BPA_30_161_10.0_ON_NM	D1_161_10.0_NM
D2	BPA_30_161_10.0_ON_2_A	D2_161_10.0_A
D3	BPA_30_161_10.0_ON_3_A	D3_161_10.0_A
D4	BPA_30_161_10.0_ON_4_A	D4_161_10.0_A
E	BPA_30_100.63_7.5_ON_A	E_100.63_7.5_A
F	BPA_30_100.63_7.5_ON_2_A	F_100.63_7.5_2_A
G	BPA_30_100.63_7.5_ON_3_A	G_100.63_7.5_3_A
E2	BPA_30_100.63_7.5_ON_4_A	E2_100.63_7.5_4_A
H	BPA_30_100.63_3.96_ON_A	H_100.63_3.96_A
I	BPA_30_100.63_11.04_ON_A	I_100.63_11.04_A
J	BPA_30_15.24_7.5_ON_NA	J_15.24_7.5_NA
K	BPA_30_186.01_7.5_ON_A	K_186.01_7.5_A

4.3 Preliminary photo-Fenton experiments and BOD analysis

Preliminary photo-Fenton experiments were performed to learn the technique and to find out the right duration of the experiment as well as effects and adjustment needs of pH and temperature. In this chapter, there are also presented preliminary BOD analysis needed in order to define the final design of experiment.

4.3.1 Preparation of the BPA solution and dilutions

BPA has low water solubility, about 120-300 mg/L depending on the source. It has a greater solubility at alkaline pH values. (BPA Global Group 2002.) BPA dissolves in alcohol-water mixture and there are also other ways for dissolving (Research Gate 2014).

It was realized that even preparing the 30 mg/L solution, BPA did not dissolve. Because presence of other substances was not desirable due to research method, it was decided to prepare the solution of 120 mg/L by heating the solution approximately up to +60ºC until BPA was dissolved. BPA solution was stored in the fridge and dilutions of 30 mg/L were made when they were needed. Dilutions for calibration curve (2.5; 5.0; 7.5; 10.0; 20.0 and 30.0 mg/L) were made using one dilution of BPA (30mg/L). Reliability of the BPA solutions used in experiments is discussed in Chapter 5.1.1.

4.3.2 BOD results of the BPA solutions - technique, the volume of the sample and the amount of the aliment

BOD analyses were implemented in accordance to the instructions of UPC laboratory using four pieces of equipment placed in two frigothermostat. BOD_5 was analyzed from solutions of BPA of different concentrations and two different sample sizes were used (Chapter 3.3). Unexpectedly there was a problem with two devices (2 and 4), which did not start measuring properly. Because this was realized in the next morning, new solutions were made and new measurement started with two devices one day later (12 samples). With the new sample solutions (428 mL), the second measurement worked properly, but the temperature of the frigothermostat was higher than +20ºC, it was not stabile and could vary several degrees during the analysis. The results of the BOD analysis are presented in Appendix 6.

Figure 1 presents the evolution of the oxygen demand during 5 days. It is clear that in the first day or two, the oxygen demand was the highest and after that it was rising more slowly. This indicates that the analysis worked properly and there were no significant problems, such as leakage of air. Two samples (0.0 yellow and 7.5 blue) indicate also that there was no bacterial activity without aliment.

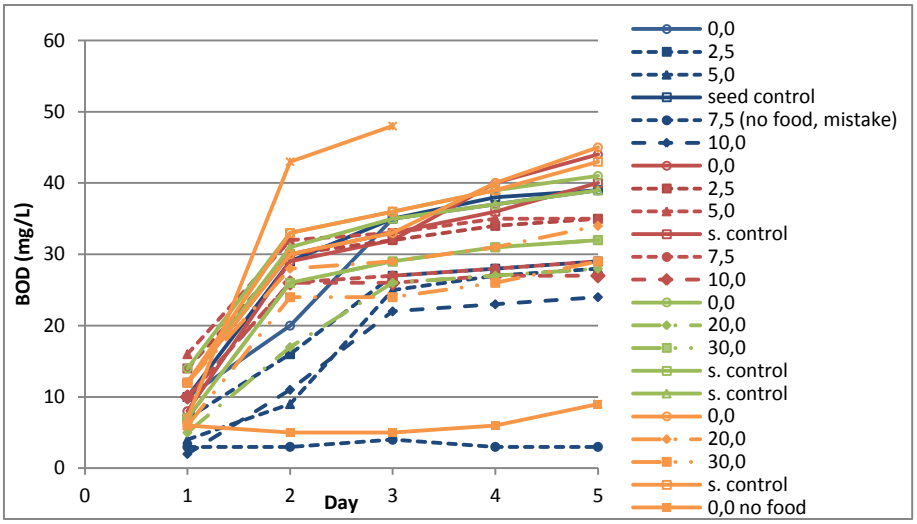


Figure 1. BOD_5 of the BPA solutions. Different pieces of equipment can be separated by the colour (Eq1=blue, Eq2 = red, Eq3 = green, Eq4= yellow). Devices 1 & 3 were in a reliable frigothermostat but the temperature in other frigothermostat (Eq 2 & 4) was not so stabile.

Based on the results of the BOD_5 analysis, it can be concluded that the sample size does not have a significant effect on the results (Figure 2). As seen in Figure 2 and Table 5, overall the sample size of 428 mL had slightly higher oxygen demand than 360 mL, apart from the sample of 30 mg/L. Higher values could be explained by the difference in temperature or the little variation caused by the fact that different solutions had to be used. In most samples the BOD_5 was a little bit higher when using devices 2 and 4 (428 mL), which can be due to a warmer temperature in the frigothermostat. Although the 30 mg/L of BPA had the opposite results. The variation between samples of same concentration was small, indicating that the method was reliable, but also that the sample size was not affecting on the results.

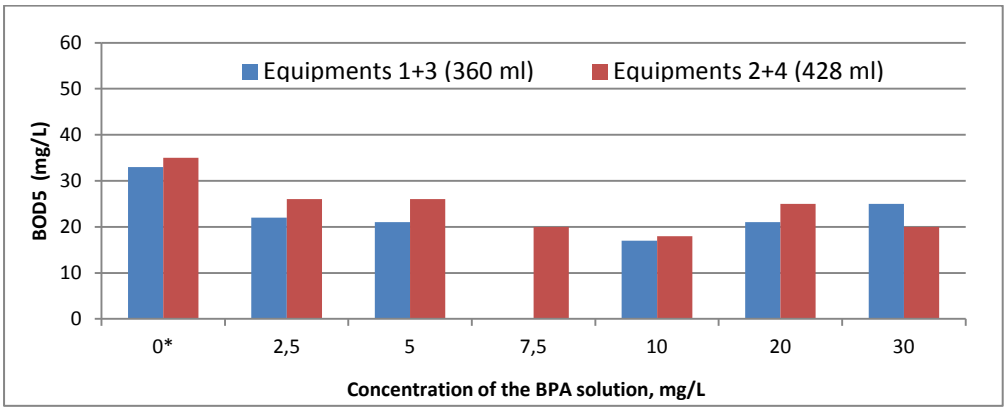


Figure 2. BOD_5 of BPA solutions (aliment 50 ppm). Comparison of different sample sizes. Devices 2 & 4 (428ml), red color. Devices 1 & 3 (360 ml), blue color.

The BOD_5 values are corrected by subtracting the oxygen demand of the 0-sample (without aliment). In case of 428 mL sample, the 0-value was based on the 0 sample in the same equipment, giving the BOD_5 value of 9 mg/L. In case of 360 mL sample, the 0-value (7mg/L) was determined based on BOD_5 values of 0-samples during the later BOD analysis (Appendix 11).

Table 5 presents the corrected values of both sample sizes as well as the mean value of them. Measured values are corrected by taking off the BOD_5 of deionised water. In Figure 3, it can be seen that the chosen amount of aliment (50 ppm) is in relation to previous results of Navarro's study. However, there is not a clear connection between the BOD behaviour when the concentration of aliment is 50 mg/L or 100 mg/L. For example with BPA concentration 7,5 mg/L, the BOD was highest when there was 100 mg/L of aliment, but the second lowest when there was 50 mg/L of aliment. There is no clear consistency with results having different amount of aliment.

Table 5. BOD_5 of deionised water. Concentration of aliment was 50 mg/L. Values below (15 and 100 mg/L) are from Navarro's study. Highlighted values of 360 ml (Eq 1+3) were used in calibration curve.

BOD_5 results and corrections	**Concentration of the BPA solution (mg/L)**								
	0 (no aliment)	**0***	**2.5**	**5**	**7.5**	**10**	**20**	**30**	**7.5 (no aliment)**
Devices 1+3 (360 ml)	-	40	29	28	-	24	28	32	3
Devices 2+4 (428 ml)	9	44	35	35	29	27	34	29	
corrected 1+3 (-blank [7])	-	33	22	21	-	17	21	25	
corrected 2+4 (-blank [9])	0	35	26	26	20	18	25	20	
Mean BOD_5	**9**	**34**	**24**	**23.5**	**10**	**17.5**	**23**	**22.5**	
Previous results, Conc. of aliment:									
15 mg/L		*7*	*11*	*10*	*-*	*9*	*-*	*10*	
100 mg/L		*54*	*-*	*46*	*63*	*56*	*-*	*49*	
**mean value of two measurements* *Obs. blank value 7 mg/L was taken from the later BOD analysis (Appendix 11)*									

As Navarro concluded (2013, 103), the low amount of aliment (15ppm) was not showing variation between samples and did not give reliable results. Due to available resources, in this study it was not possible to research more about the effects of different amounts of aliment, so the preliminary 50 ppm was chosen from the middle of Navarro's values.

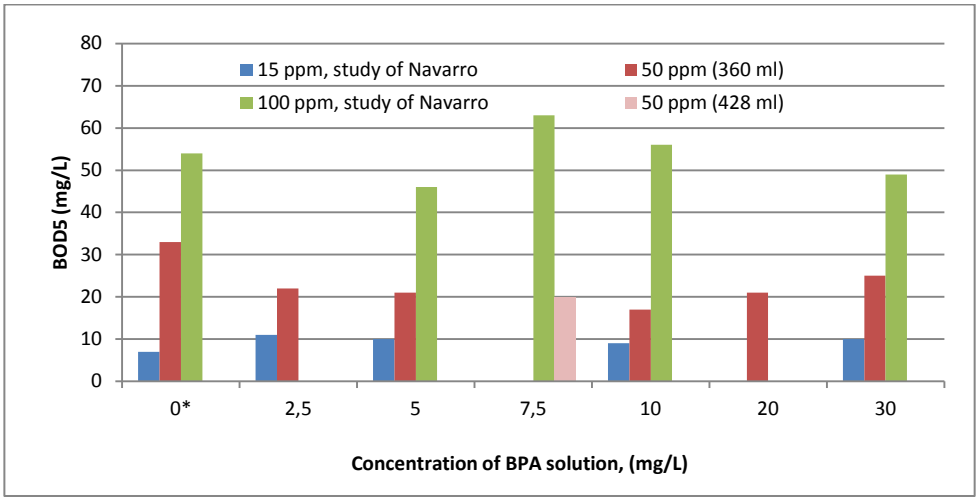


Figure 3. BOD_5 of BPA solutions in relation to the quantity of aliment. 50 ppm was chosen for the BOD analyses.

To compare the effects, half (25 mg/L) and double (100 mg/L) concentrations of the aliment was planned to do with concentrations of 10 mg/L and 20 mg/L of BPA. Because some first results were lost due to technical problems explained above, at the end comparative results included only one sample of both concentrations of aliment (25 mg/L and 100 mg/L) added in the solution of BPA (20 mg/L)(Table 6). Unfortunately Navarro did not have results in this concentration of BPA for comparison. However, the sample of 100 mg/L concentration, resulted to "OFL" after 3 days of measurement, which means that values were above the permitted measurement range. In this case assumingly the amount of the aliment would have been too high, even though experimental error is also possible. Therefore replicates would have been needed to assure the comparison of different amounts of aliment.

Table 6. The BOD_5 of BPA solution (20 mg/L) using different amount of aliment.

Concentration of the aliment	**BOD_5 (mg/L)**
25 mg/L	22
50 mg/L	21
100 mg/L	day 3: 41 / OFL

Preliminary tests with BOD_5 indicated that the chosen concentration of aliment (50 mg/L) showed the variation between samples in a better way than Navarro's low concentration (15 mg/L). It was also found that the replicates were reliable even though sample size was different, so the concentration of 50 mg/L was chosen for experiments. Since the volume of the sample did not significantly effect on results, it was chosen to use 360 mL sample size to minimize the need of the solution in the experiments. For consistency, also the values of 360 mL samples were used in calibration curve instead of mean values, which could have been one option.

Based on the results of these preliminary BOD analyses, biodegradability of the BPA solutions of different concentrations was also evaluated. These results were needed in order to be able to have comparative data for the experiments. The results are presented in Chapter 5.2.2.

4.3.3 Duration of the experiment

First preliminary experiments took 60 minutes, but soon it was realized that it was not enough to see that the photo-Fenton process had stabilised (Figure 4). It was decided to extend the duration to 90 minutes, when the TOC curve indicated that the process has reached an almost steady state.

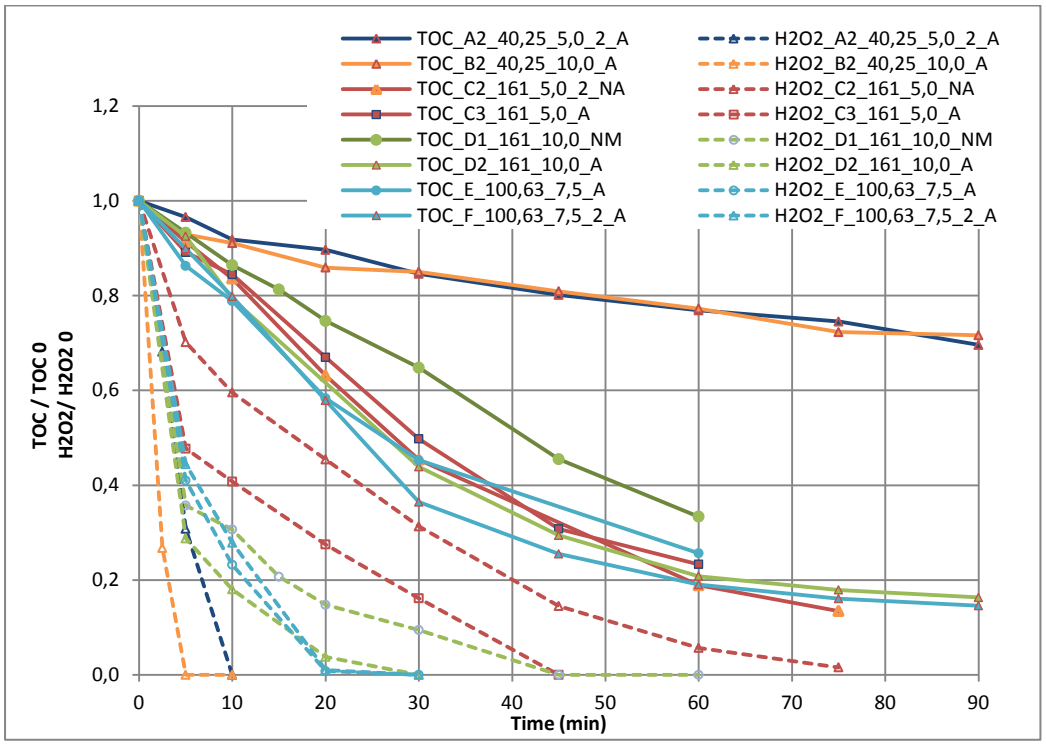


Figure 4. TOC and H_2O_2 of the preliminary experiments compared to the later experiments. The duration of 60 minutes was not enough to be sure that the photo-Fenton process has reached a steady state, so experiments were extended to 90 minutes.

4.3.4 The effects of pH & temperature

During the preliminary experiments, it was noticed that the heating effect of UV-light was significant after some experiments were implemented. The first experiments of the day were not problematic, but after a few hours and some experiments, the temperature of the solution began to rise. The water bath was able to warm up the solution inside the container and keep it stable, but it could not cool it down when the temperature got higher because of the UV-light.

The solution was first cooled down by adding ice into the water bath, which made the temperature drop down but quite unpredictably. Controlling the temperature by adding ice in the middle of the experiment was realized too unpredictable and disturbing for the process. Figure 5 presents how the significant cooling down also slowed down the photo-Fenton process and resulted higher TOC at the end of the experiment. Similar behaviour could be seen in two different experiments, B and the central ones (E-G).

In the lower graph (Figure 5), the temperature of the two first experiments (E, F) was quite similar, +26ºC ± 1.5 . In the beginning of the third experiment, the temperature was also +26ºC, but soon it began to rise and some ice was added in the water bath to cool down the BPA solution in the container. After 10 minutes, temperature was +25ºC and after 20 minutes it dropped to +24,5ºC. After 30 minutes, temperature was back to +25ºC and stayed quite stable until 75 minutes (+25,2ºC). At the end, after 90 minutes, the temperature was back to +26ºC. In the graph, it is clearly seen that cooling down disturbed the process and the curve G is higher than the other ones.

The graph above shows similar behaviour, because B2 was cooled down and at 30 minutes point the temperature dropped from +27,0ºC. to +26,0ºC and the curve is higher than the one that was not adjusted.

To confirm the result, the experiment was repeated once more (E2) without interfering the temperature. Experiment E2 was adjusted only in the beginning, which did not disturb the process. In this 4th experiment,

the temperature rose from +26,2ºC to +27ºC and the curve was similar to the first ones. For the final results the 3rd experiment (G) was excluded (Appendix 10).

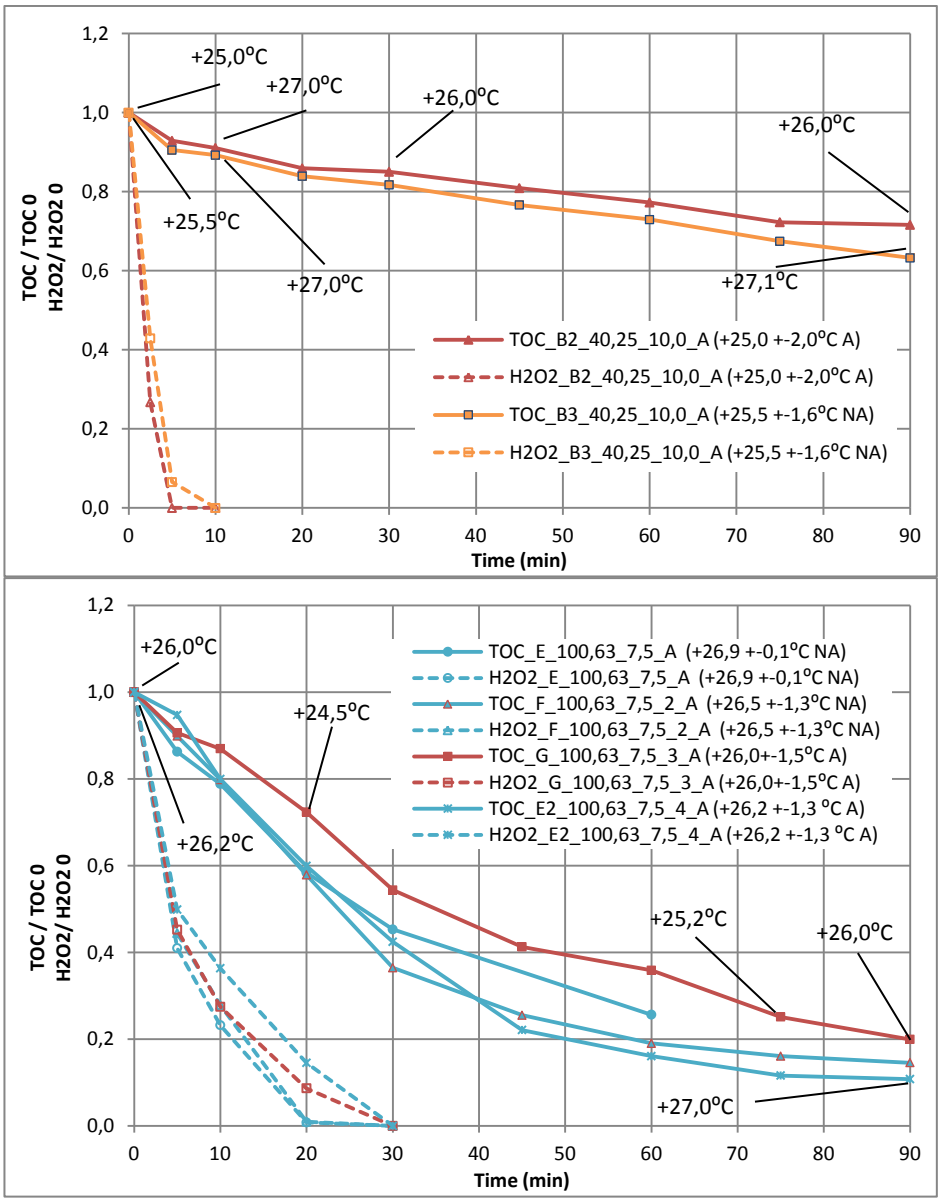


Figure 5. Effects of the adjusted temperature on the photo-Fenton process. Graph above presents the results of the experiment B and the graph below consists central experiments (E-G).

Based on the observations, it was decided not to interfere the temperature radically in the middle of the experiment and let the temperature rise in order to not slow down the process. Always in the beginning of the experiment, the ice was added to cool down the solution, but unfortunately

in some experiments later on the day it was not possible to control the temperature well enough. Although the variation of the temperature may effect on the results and make the experimental characters difficult to replicate, it was concluded that the "naturally" rising temperature gives still more accurate and replicable results than cooling down manually. The temperature was monitored and the data is presented in Appendix 10.

In ideal conditions, the temperature stays stabile. But Figure 6 presents that even if the temperature raises 1-2ºC during the experiment, it does not necessarily have remarkable effect on the process or TOC results. However, also in this case the warmer conditions (C2) gave better TOC result in the end. Experiment C2 was not adjusted, and in the beginning the temperature was higher than in case of C3. It was one of the last experiments, when the surroundings were heated by the UV-light. C3 was performed in another day, cooled down only in the beginning and the temperature was rising only 1ºC. TOC of both experiments is still quite similar, although higher temperature lead to slightly better result in the end.

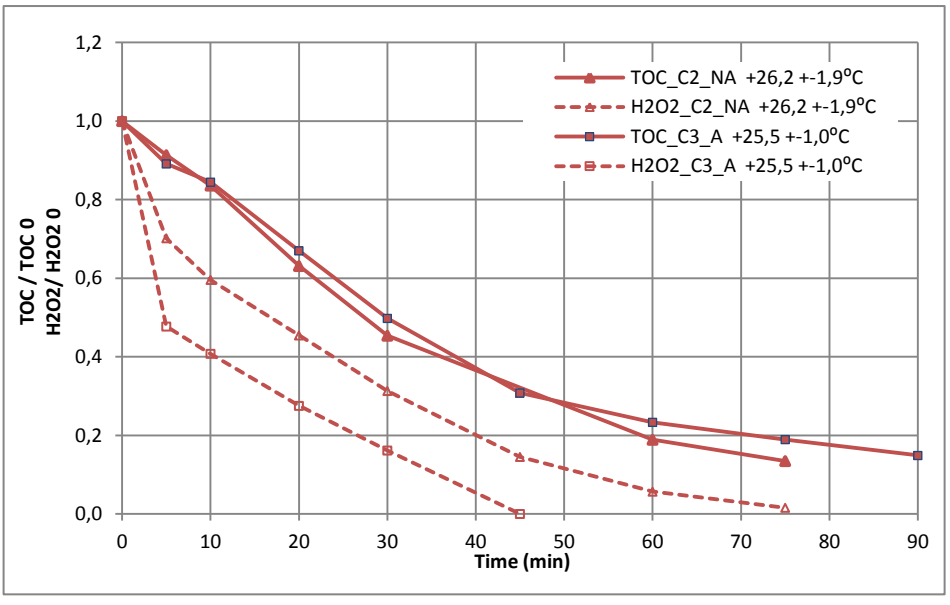


Figure 6. Effects, if the temperature was adjusted only in the beginning of the experiment.

As a conclusion, the results seem to indicate that rising temperature does not effect remarkable on the TOC. But as seen in Figure 5, cooling down and interfering the process has undesirable effects and can cause unrepeatable conditions.

Based on the preliminary tests, the temperature in the beginning of the experiment was adjusted to +25ºC and after it was allowed to rise. The variation among the experiments was between 0.0 - 2.4ºC. Among the final experiments it can be concluded, that the temperature variation was $+25^{0}\mathrm{C} \pm 2.0$, apart from one experiment, which had higher variation. Experiment H_100.63_3.96_A had the variation of 2.4ºC.

The effect of adjusted pH was not as clearly seen as the effects of temperature. Usually pH dropped in the beginning below 2,8 and was adjusted with NaOH. Even though in some cases it seemed that adjusting the pH after first 5 minutes was causing slight changes in the graph, the results were not consisting and the small variation can as well be part of the process or caused by some other factor. The pH was adjusted 0-3 times during the experiments to keep it at pH 2.9 ± 1 (Appendix 9).

4.4 Blank experiments

The experiments with blank samples were not performed in this study, because the results of Navarro's study (2013) could be applied. As seen in Figure 7, in the blank samples alone the degradation of BPA as well as mineralization are minor. The results show that use of reagents and UV-light is justified in order to remove BPA from the water.

Navarro's study verifies that mineralization is not achieved using the reagents separately. The degradation of BPA remains zero, however 10% degradation is achieved during 90 minutes by adding UV light. By adding H_2O_2, the 30% degradation is achieved, but the TOC is the same as in the beginning. This indicates that even though BPA degrades, there are some organic intermediates present.

Furthermore, when maximum stoichiometric H_2O_2 is used (161 mg/L) both the Fenton and the photo-Fenton remove the BPA quickly, but the difference lies in the mineralization. In the Fenton reaction, the limiting factor is the reduction of the Fe(II), and the mineralization is not achieved as in the photo-Fenton. Adding UV light (photo-Fenton) with the ratio 161 mg/L H_2O_2 and 10 mg/L of Fe(II), the mineralization is significant.

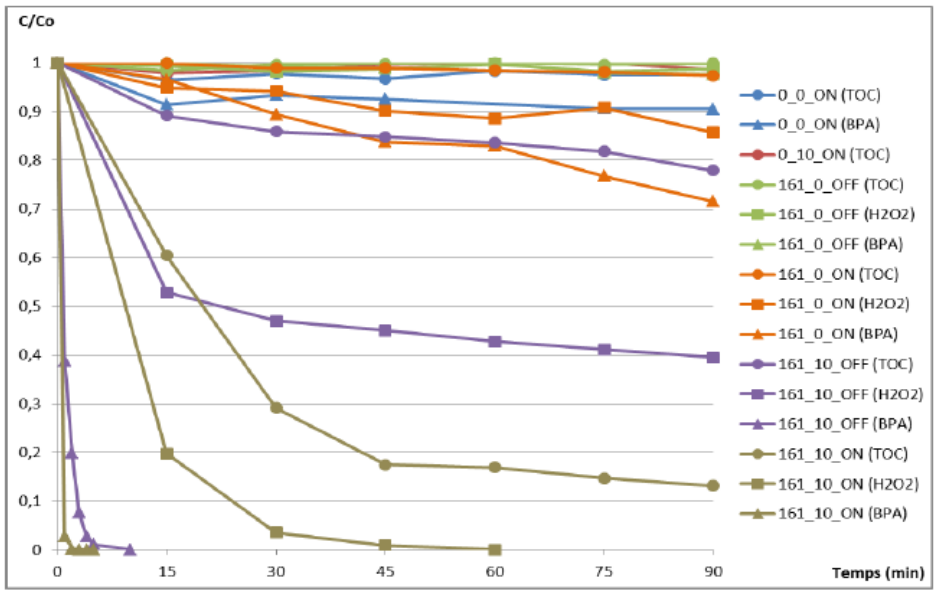


Figure 7. Evolution of the TOC (point), H_2O_2 (square) and BPA ratio (triangle) on blank samples in comparison to the experiments including reagents. The difference between Fenton (light OFF) and photo-Fenton (light ON) reaction can also be seen. (Navarro 2013, 83.)

4.5 The design of the experiment

4.5.1 Preliminary experiments and analysis

Preliminary experiments include the first experiments that were performed in accordance with the following design of experiment (4.5.3). The purpose of the preliminary experiments was to define the duration of the experiment and observe the effects and adjustment needs of the temperature and pH. Preliminary BOD analyses were also needed in order to learn the technique, get the calibration curve and define the sample size.

The results of the preliminary experiments are presented in Chapter 4.3. Based on these results, the DOE was modified in order to get more accurate results.

4.5.2 The design of the experiment (DOE)

Concentration of the reagents

In Navarro's study, different quantities of H_2O_2 and Fe(II) were used in photo-Fenton experiments and her conclusions are presented in Figure 8. Considering the errors, there are many experiments that overlap. Inside the square are the best results of Navarro's experimental design (green marks). These one are 161_11,04_ON, 161_7,5_ON and 201,25_10_ON, equal to 1.00/5.36/0,37; 1.00/5.36/0.25 and 1.00/6.70/0.33 (BPA/H_2O_2/Fe(II)). (Navarro 2013, 88.)

In Navarro's study, the minimum H_2O_2 quantity was defined as 120,75 mg/L and the maximum 201,25 mg/L, when the central value according to the DOE was 161 mg/L. Initial value of 161 mg/L was based on the stoichiometric concentration of H_2O_2 needed to mineralize the 30 mg/L of BPA. This concentration was calculated based on the following reaction:

$$C_{15}H_{16}O_2 + 36H_2O \rightarrow 15\,CO_2 + 44H_2O \qquad (12)$$

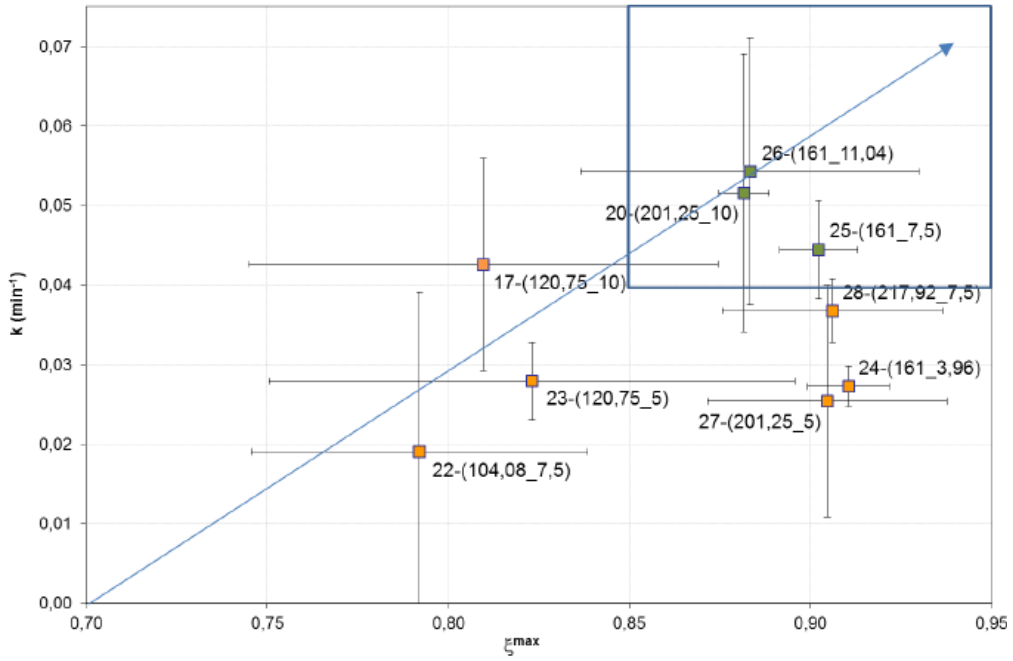


Figure 8. Presentation of the results of Navarro's study. Optimized conditions regarding the k and ξ^{max} including the error bars. (Navarro 2013, 88)

In addition to DOE, the lowest concentration of H_2O_2 studied was 80,5 mg/L and the highest 270 mg/L. According to Katsumata et al. (2004), the best value for degradation in their operating conditions was 9/0.25/1, which in Navarro's study is equal to H_2O_2 concentration of 270 mg/L and 7.5 mg/L of Fe(II). (Navarro 2013, 77.)

In Navarro's study, the minimum quantity of Fe(II) was 5 mg/L and the maximum 10 mg/L. According to DOGC (Diari Oficial de la Generalitat de Catalunya) no. 3894-29 / 05/2003 the discharge limit for wastewater treatment plant is 10 mg/L for iron, which was defined as maximum value. (Navarro 2013, 77.) For this reason, increasing the iron quantity was not desirable either in this study. Therefore, the concentrations of iron are kept similar, which also makes the results more comparable with previous study (Table 8).

Based on Navarro's results (Figure 8), in this study the stoichiometric 161 mg/L was chosen as maximum quantity of H_2O_2. In order to find out the best possible ratio of the reagents and minimize their amount and costs, the minimum value was defined as one quarter of the stoichiometric value. (Table 8.)

Table 8. Concentration of H_2O_2 and Fe(II) for the experiments.

Variables	**maximum**	**minimum**	**central**
Fe(II) (ppm)	10	5	7.5
H_2O_2 (ppm)	161 (Stoichiometric)	40.25 (1/4* Stoichiometric)	100.63 (Central)

Final design of the experiment

When the initial experimental design was defined, the final design was in order to get more accurate results. The design of 2^2 star with three central points was used, which was also used in Navarro's study. The reason for choosing this design was the extension of the analyzed area that gives a significant decrease in errors in calculating the response surfaces. Figure 9 presents the design, where each axes represent a variable and the values -1 and 1 are representing the minimum and maximum value selected from the initial design. (Navarro 2013, 80; Barros et al. 1995.)

Table 9 shows the concentrations of the reagents according to the coding. In ideal situation, the duplicates would have been performed in all experiments. Because the time for the project was limited, the experiments A-D were prioritized and the ones giving statistical accuracy (H-K) were planned to be duplicated only, if there was time left. For the centrals there were 3 values. Finally, because of some excluded samples, there was also need for extra duplications and the experiments H-K were left without duplications.

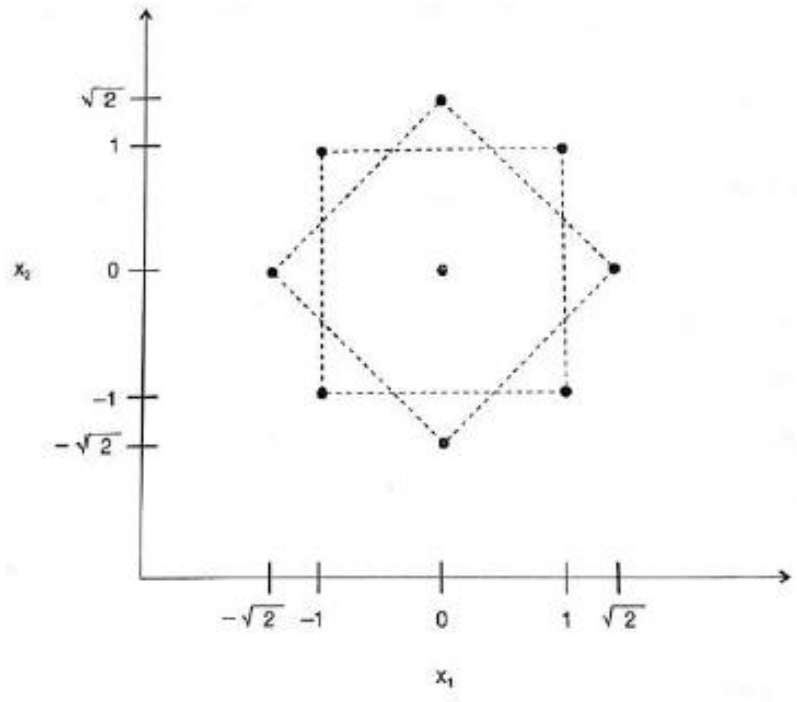


Figure 9. Presentation of the 2^2 star design with the central point. (Navarro 2013, Barros & all. 1995).

Table 9. Concentrations of the variables and the codification.

Assay	Codified values		Variables levels	
	Fe(II)	H_2O_2	Fe(II)	H_2O_2
A	-1	-1	5.00	40.25
B	1	-1	10.00	40.25
C	-1	1	5.00	161.00
D	1	1	10.00	161.00
E	0	0	7.50	100.63
F	0	0	7.50	100.63
G	0	0	7.50	100.63
H	-1.414	0	3.96	100.63
I	1.414	0	11.04	100.63
J	0	-1.414	7.50	15.24
K	0	1.414	7.50	186.01

Toxicity and biodegradability

Navarro's results show that both iron and light are needed in the photo-Fenton reaction to degrade BPA. Under these conditions, BPA is degraded in few minutes, in all studied cases in less than 10 minutes. (Table 10.) However, depending on concentration of reagents, up to 60 or 90 minutes is needed until the mineralization is complete and TOC stabilized on low level (Figure 7).

Katsumata concluded also that BPA was degraded in 9 minutes under his experimental conditions, and six intermediate products were identified. However, the percentage of the sum of the six intermediates and remaining BPA concentrations to the initial BPA was 93 %, which indicates that remaining percentage may consist unidentified intermediates. It should also be noted that the reason can be analytical error and/or loss of the intermediates during the analytical process. (Katsumata 2004, 304.)

Existing by-products may be more toxic than BPA itself (Katsumata 2004, 301; Navarro 2013), and one purpose of this study is to evaluate the evolution of toxicity during the photo-Fenton experiment. For this purpose TOC, H_2O_2 and toxicity are determined from the samples taken during the experiment. According to Pérez-Moya et al. (2007), H_2O_2 remaining in the sample will have an effect on toxicity analysis, so it should be known when all of it has been consumed. Consumption of H_2O_2 is monitored during the experiments.

Navarro has concluded that BPA is not biodegradable with the concentration studied (30 mg/L). Micro-organisms could not degrade BPA, but neither BPA affected the activity if micro-organisms had an alternative source of food. According to Navarro, BOD analysis of the samples (Table 10) after the photo-Fenton process had been applied were not reliable. Unreliability existed because of practical and technical issues and the variation that could have been caused by experimental error.

Table 10. Some of Navarro's results, degradation of BPA and biodegradability of the sample (Navarro 2013, Appendix p. 46). Biodegradable samples are highlighted as well as the two experiments having same conditions than in this study. It should be noted that Navarro concluded BOD test had some unreliability.

Experiment	Time of exp. (min)	[BPA,ppm] ; (min)	Biodegradability	
			Sample	Sample +Aliment
161_0_OFF_1	90	30 (90)	-	-
161_0_ON_1	90	21 (90)	-	-
80.5_5_ON_1	90	-	no	no
80.5_10_ON_2	225	0 (4)	no	no
104.08_7.5_ON_2	180	0 (6)	no	yes
120.75_5_ON_1 (_2)	300 (180)	0 (10)	yes (no)	yes (no)
120.75_10_ON_2	360	0 (3)	no	no
161_3.96_ON_1	300	0 (5)	-	-
161_5_ON_2	180	0 (10)	no	no
161_7.5_ON_3	180	0 (5)	no	no
161_10_ON_1	90	0 (3)	no	yes
161_11.04_ON_2	180	0 (4)	no	no
201.25_5_ON_2	180	0 (8)	no	yes
201.25_10_ON_2	180	0 (4)	yes	yes
217.92_7,5_ON_2	180	0 (5)	no	no
270_7.5_ON_1	170	0 (3)	-	-

Based on theoretical TOC value of BPA (24,14 mg/L, see equation 6, page 23), the expected BOD values were calculated in order to compare them to Navarro's results and to plan this study. Table 11 shows that lower concentrations probably will be biodegradable, and the concentration 30 mg/L is not, but the uncertainty lies in the concentrations in the middle.

In this study, the purpose is to get results and reassurance about the biodegradability of the end solutions after BPA is treated with the photo-Fenton. Based on preliminary analysis (Chapter 4.3.2), the chosen sample size for BOD tests was 360 mL and the concentration of the aliment 50 mg/L. Because of the limited time and capacity of the equipment, it was decided to analyze BOD of each experiment once and for statistical

accuracy the three centrals. To get more accurate results, the replicates of all could have been performed. Finally, two replicates were performed in addition to initial DOE.

One option could have been also to study the biodegradability during the photo-Fenton experiment, but it was considered that first should be more reliable information about the end solutions. The sample size should also have been increased in that case. In this study, it was decided to concentrate on the evolution of toxicity and biodegradability of the end solutions in different concentrations of reagents (H_2O_2 and Fe(II)).

Table 11. Expected BOD_5 values to indicate degradation of BPA partially or totally, based on the theoretical TOC value. Theoretical value is compared to Navarro's results (2013, 62) in order to make hypothesis of the planned BOD analysis.

BPA (mg/L)	$TOC_{theor.}$ (mg/L)	$COD_{theor.}$ (mg/L)	Expected BOD partially	Expected BOD totally	Assumption of biodegradability
0	0.00	0.00	0.00	0.00	Yes (7)
2.5	2.01	5.37	2.15	3.22	Yes (11)
5	4.02	10.73	4.29	6.44	Yes (10)
7.5	6.04	16.10	6.44	9.66	-
10	8.05	21.46	8.58	12.88	Partially (9)
20	16.09	42.92	17.17	25.75	-
30	24.14	64.38	25.75	38.63	No (10)

5 RESULTS AND DISCUSSION

5.1 TOC

The TOC results and the data of the experiments are presented in Appendix 12. In the next Chapter 5.1.1, the reliability of the method is explained as well as several BPA solutions used. In Chapter 5.1.2, the actual TOC results of the photo-Fenton experiments are presented and discussed.

5.1.1 TOC of the BPA solutions and dilutions

As explained in Chapter 4.3.2, preliminary BOD analysis had to be carried out again with devices 2 and 4. Because of this, new dilutions of BPA had to be made for these analyses. In this case it could have been possible that there are differences in results because the samples of 360 mL were not exactly from the same solution than the new 428 mL samples. To make sure the solutions were equal, TOC analysis were taken from both of the solutions. It was also more accurate for the calibration curve to have two TOC measurements instead of one.

As seen in Figure 10 and Table 12, the TOC of two different solutions used in dilutions do not have significant variation and are reliable. Figure 11 shows that the R squared value of the regression line is 0,99 indicating that the analysing method is reliable.

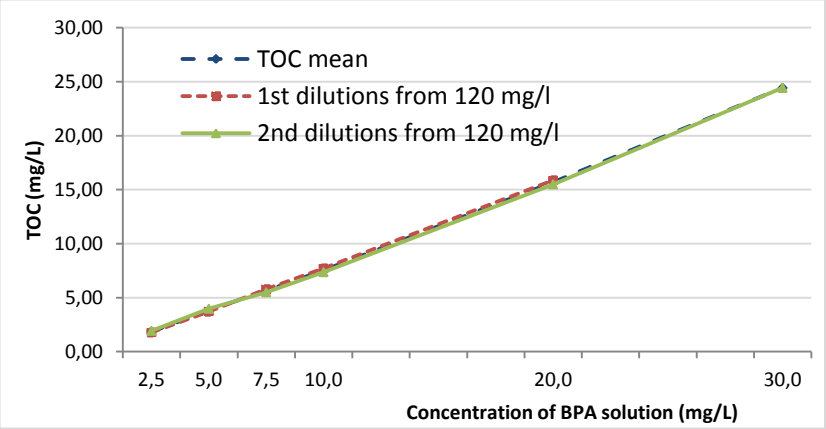


Figure 10. TOC of the dilutions of BPA (2.5; 5.0; 7.5; 10.0; 20.0; 30.0 mg/L) made of different solution (120 mg/L) is quite equal. The mean value is used as calibration curve when analysing the results of the experiments.

Table 12. Total carbon (TC), inorganic carbon (IC) and total organic carbon (TOC) of two different solutions of BPA. In most cases TOC mean value is slightly below the theoretical value, which is acceptable. Curves are presented in figure 10.

Conc.	1st dilutions from 120 mg/L solution			2nd dilutions from 120 mg/L solution			TOC	Theoretical TOC
	TC	IC	**TOC**	TC	IC	**TOC**	mean	
2.5	2.530	0.753	**1.78**	2.766	0.840	**1.93**	1.85	2.01
5.0	4.417	0.709	**3.71**	4.984	1.010	**3.97**	3.84	4.02
7.5	6.554	0.769	**5.79**	6.290	0.791	**5.50**	5.64	6.04
10.0	8.347	0.634	**7.71**	8.124	0.764	**7.36**	7.54	8.05
20.0	16.670	0.795	**15.88**	16.510	1.015	**15.50**	15.69	16.09
30.0				26.200	1.766	**24.43**	24.43	24.14

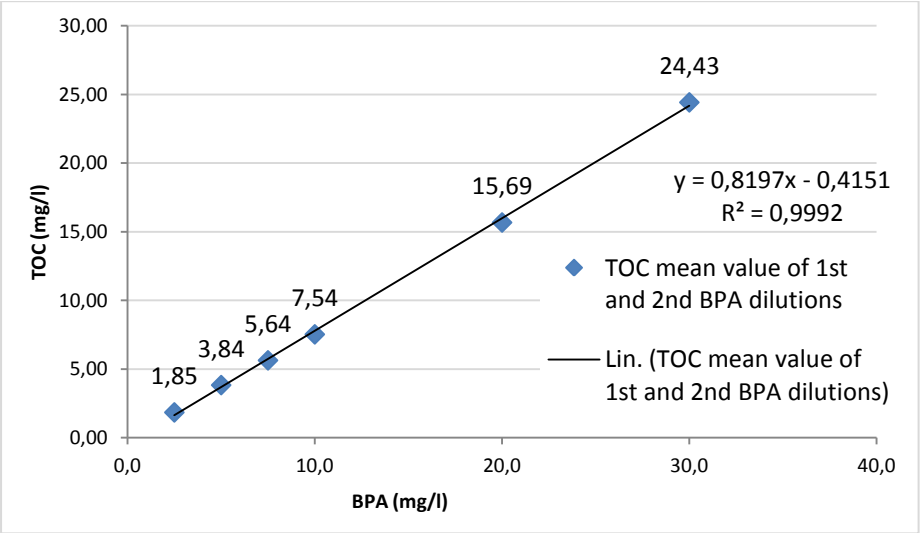


Figure 11. TOC mean value of the 1st and 2nd dilutions of BPA (2.5; 5.0; 7.5; 10.0; 20.0; 30.0 mg/L). The mean values are used in calibration curve when analysing the results of the experiments. The R squared value is 0.999, which is a very good fit of the line to the data. This indicates that the analysis method is reliable.

The BPA solution of 120 mg/L was prepared in order to make the dilutions (30 mg/L) needed in the experiments. At the end, several dilutions were made, because of the limited space in the fridge. It was more practical to keep only the solution of higher concentration in the fridge and prepare dilutions when needed. In Figure 12, the TOC of the all BPA dilutions (30mg/L) used in the experiments is presended. TOC of the solution II was significantly different, possibly due to a contamination of the solution. Experiments where this solution was used were excluded. Appendix 8 presents which solution was used in each experiment.

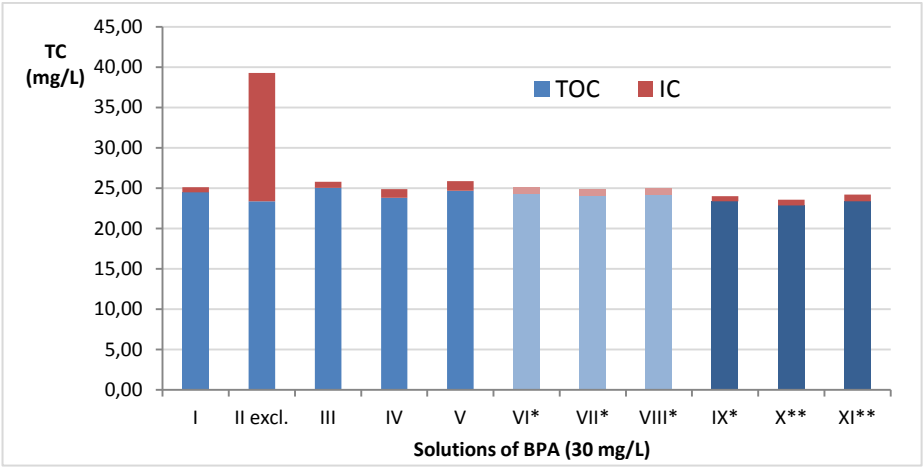


Figure 12. TOC of the BPA solutions (30mg/L) used in experiments. Dilutions I-V were made from the same BPA solution (120 mg/L), VI-VIII from the second (*) and IX-XI from the third solution (**). Experiments where solution II was used were excluded.

Table 13. TOC of BPA solutions (30mg/L) for experiments, diluted from [120 mg/L] of BPA solution. Solutions I-V were made in one solution, VI-IX another one (*) and the last solutions X-XI had the third solution (**).

Date	Solution no	TC	IC	TOC
13.10.	I	25.12	0.674	24.45
20.10.	*II excl.*	*39.28*	*15.93*	*23.35*
22.10.	III	25.76	0.751	25.01
22.10.	IV	24.87	1.098	23.77
28.10.	V	25.84	1.177	24.66
28.10.	VI*	25.1	0.826	24.27
30.10.	VII*	24.81	0.744	24.07
31.10.	VIII*	24.99	0.757	24.23
11.11.	IX*	23.99	0.646	23.34
13.11.	X**	23.57	0.735	22.84
18.11.	XI**	24.17	0.808	23.36
	Mean TOC (II excluded)			**24.00**

5.1.2 TOC values of the experiments

In addition to preliminary experiments, 14 experiments were performed (Figure 13). All experiments were duplicated and the mean value calculated, except the experiment A2 (40.25_5.0). The solution used in this replicate was contaminated, and because the experiment was not the relevant ones (TOC remained high) it was not repeated having only limited time to use for all laboratory work. The experiments for statistical accuracy (J-K) were also performed only once, but the most important central experiments were carried out three times according to DOE. The mean values of each experiment are presented in Figures 14-16.

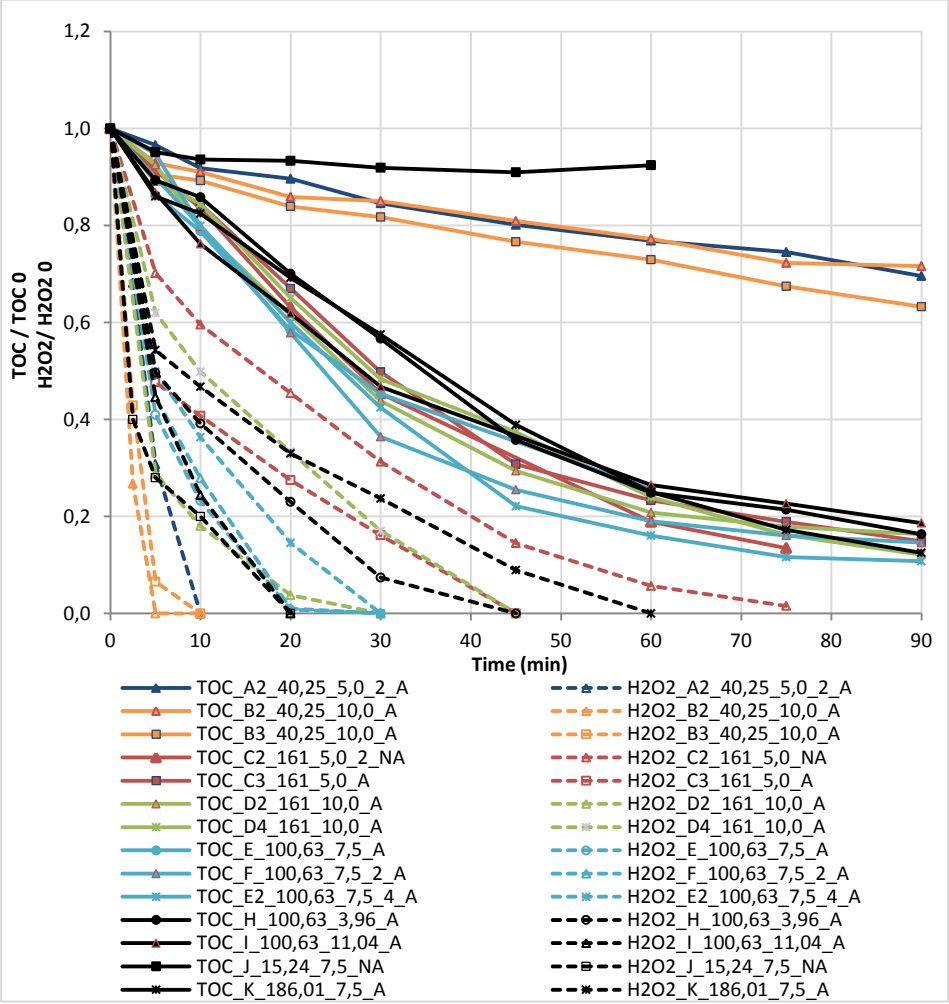


Figure 13. TOC and H_2O_2 values of all the 14 experiments after some preliminary or inaccurate ones were excluded. In total, 20 experiments were performed.

The results show that in the experiments A (40.25_5.0) and B (40.25_10.0) the small amount of H_2O_2 is consumed fast and the higher amount of Fe(II) does not have significant improvement. It is clear that more hydrogen peroxide is needed to achieve better degradation rate. Experiments C (161_5.0) and D (161_10.0) are very close to each other, but at the end the central experiments E+F+E2 (100.63_7.5) seem to have better degradation rate with less amount of reagents. Figure 16 shows that increasing the amount of Fe(II) into 11.04 mg/L from 10.0 mg/L, improves the degradation process in the beginning, but overall it does not give better results. When decreasing the Fe(II) concentration down to 3.96 mg/L, the TOC in the end is also nearly the same than having more iron with same amount of H_2O_2 (100.63mg/L). Although it should be noted that experiment H (100.63_3.96) had quite high variation of temperature ($+\ 25^0\mathrm{C} \pm 2.4$), which may have lead to slightly better results.

The experiment K (186.01_7.5) has almost similar curve compared to the H(100.63_3.96), which indicates that just simply increasing the amount of both reagents do not improve the process significantly, although the TOC at the end of K is slightly better. It should be noted that only during the experiment H the temperature rose 2,4ºC but the other experiments I-K were quite stable and there was no need to adjust the temperature ($\pm 0{,}7^0C$) (Figure 15). Because of this, the results of H may seem slightly better than they should be. In most of the experiments (A-H), the temperature rose slightly, so in these last experiments I-K the temperature was exceptionally stable, which may also result a bit lower results. Overall the conditions in most experiments were similar, and the rising temperature did not have significant role (The effects of the temperature is discussed in Chapter 4.3.4, Appendix 10).

More importantly, the right ratio of H_20_2 and Fe(II) matters when trying to find best results of the process. However, adding iron, does not have so important role than finding the best concentration of H_2O_2. Comparing experiments E+F+E2 (100.63_7.5) and I (100.63_11.04), increasing iron to conditions of central experiments (E+F+E2) did not improve the

process. Actually it is the opposite and the centrals with less iron are better than experiment I(100.63_11.04) after 15 minutes and stay better until the end. After 30 minutes C(161_5.0) and D(161_10.0) are both giving better results than I(100.63_11.04), but still do not reach the level of centrals.

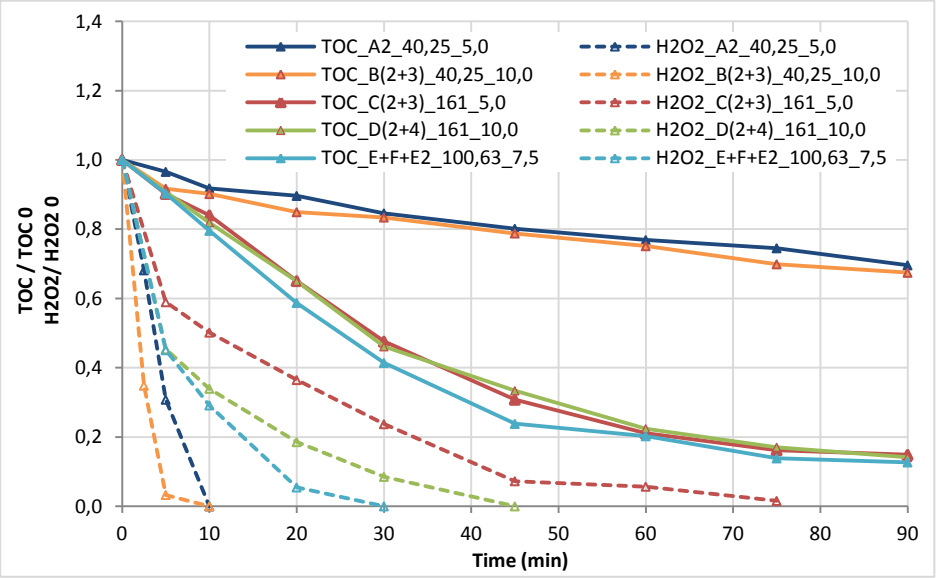


Figure 14. The mean TOC and H_2O_2 values of experiments A-F (14 experiments all together). The code (inside the brackets) informs which experiments are used to calculate the mean value.

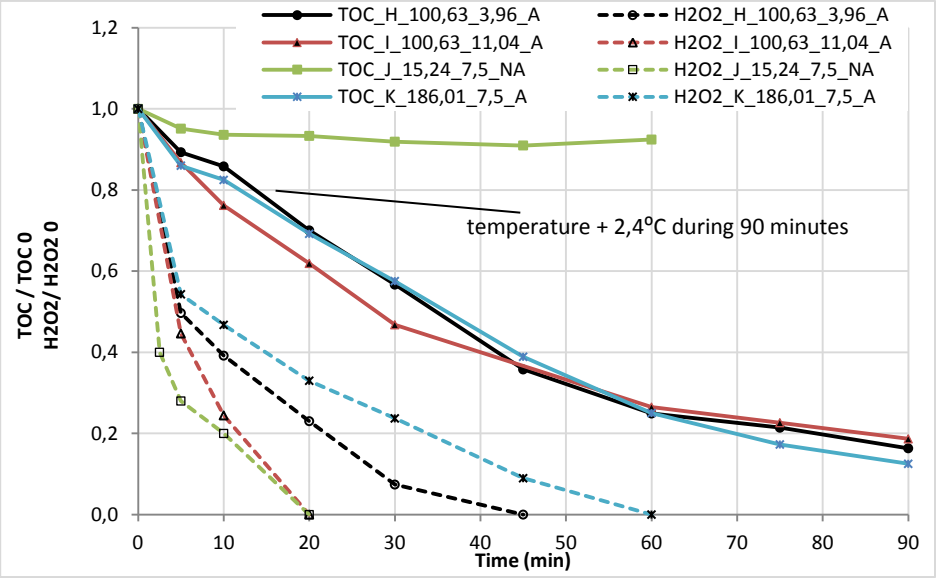


Figure 15. TOC and H_2O_2 values of the experiments H-K and the possible effect of the temperature (no replicates).

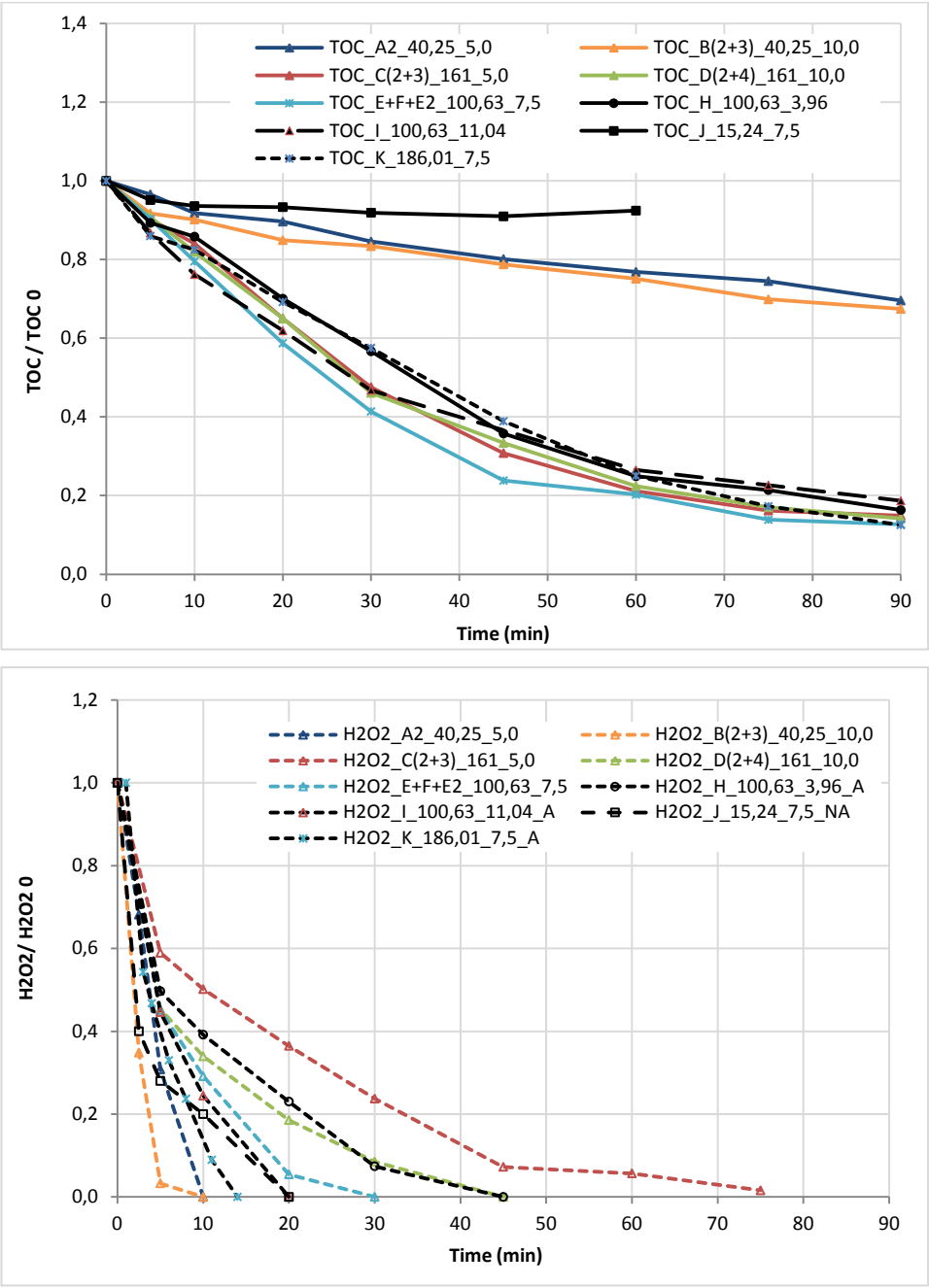


Figure 16. The mean TOC and H_2O_2 values of all experiments (A-K). The data and graphs of each separate experiment are presented in the Appendix 12.

Furthermore, exceeding the stoichiometric amount of H_2O_2 does not improve the process. It could be assumed that K (186.01_7.5) would give better results than E+F+E2 (100.63_7.5) or C (161_5.0), because K consists more **both** reagents. The results seem to indicate that exceeding the maximum stoichiometric H_2O_2, there are undesirable reaction caused

by hydroxyl radicals. Therefore the best results would be between the central and D experiments (less than 161 mg/L H_2O_2).

These results are mostly consistent with Pérez-Moya, Navarro, Mansilla & Graells (2014, 3), who concluded that increasing Fe(II) load does not provide any improvement when sub-stoichiometric H_2O_2 is used (in their study 104.08 mg/L). In their results, when stoichiometric H_2O_2 (161 mg/L) was used, increasing Fe(II) concentration from 3.96 mg/L to 11 mg/L increased the reaction rate 50% (while mineralization rate stayed similar). There was also no difference between the concentrations 3.96 mg/L and 5 mg/L, when H_2O_2 concentration was 161 mg/L (Navarro 2013, 89). In this study, there was no clear difference between experiments C(161_5.0) and D(161_10.0), so adding iron did not make a big difference. Although it was realized that E+F+E2(100.63_7.5) gave almost the same result at the end and was proceeding better already after 10 minutes.

Overall already after 60 minutes, the TOC in experiments C, D and E+F+E2 is very similar (Figure 16), even though earlier in the latter one the degradation was faster. After 90 minutes, the results are still quite similar, and TOC of E+F+E2 is slightly better than in the other ones. To be able to see the best results more clearly, the concentration of H_2O_2 was compared to the TOC results and the speed of the process (Figures 17 and 18).

Modelling would have been appropriate way to analyze and present the best results regarding the effectiveness and minimum usage of reagents. Due to the available time and possibilities, modelling was not an option, and therefore these quantities are presented visually in Figures 17 and 18. It is not likely that the TOC changes remarkably after 90 minutes even if the duration had been extended, so it is justified to use 90 minute value finding the best result. As the results show, the H_2O_2/Fe(II) relation is important because it effects on the total mineralization and the speed of the reaction.

In Figure 17, it can be seen which experiments offer the lowest TOC in comparison to H_2O_2/Fe(II) relation / amount of H_2O_2. The amount of H_2O_2 is mainly affecting the costs of the process, because it is the main reagent as Fe(II) can be considered as catalyst. Because C and D are very close to each other, it can be assumed that also Fe(II) concentration of 7,5 mg/L would have given the same result. The amount of H_2O_2 is clearly more important than the relation of reagents, if looking for the lowest costs of the reagents. Furthermore, even the K gives slightly better TOC, it is much slower (Figure 18) and not available choice considering the benefits and costs.

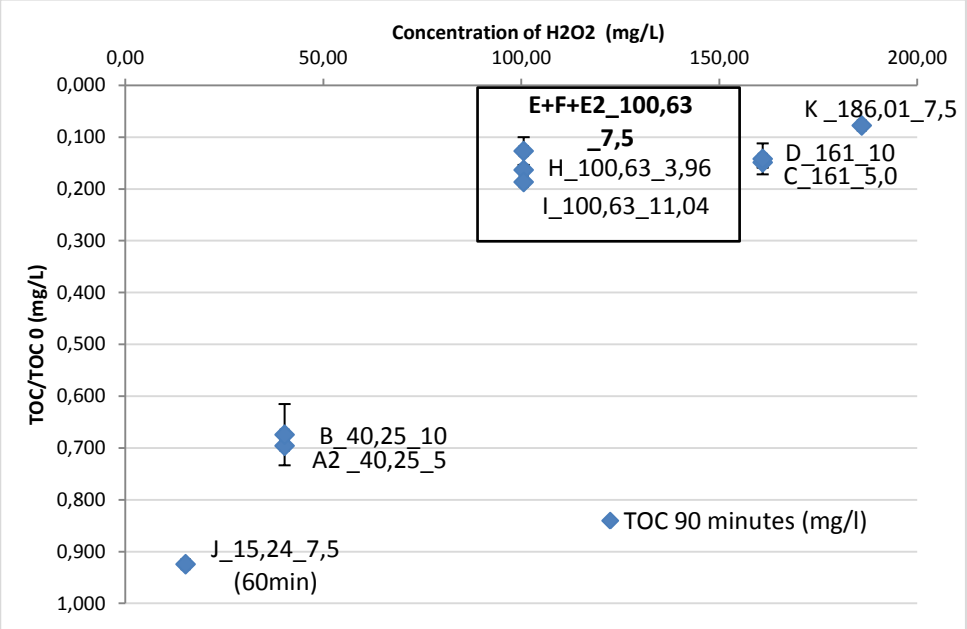


Figure 17. The best results for photo-Fenton process after 90 minutes considering the usage of H_2O_2, which is the main reagent affecting the costs of the process. SD was less than 0,06 in all cases there were replicates, values A and H-K did not have replicates.

In Figure 18, the best results according to the speed of the reaction are presented. The speed is calculated during the first 20 minutes, which shows the reaction rate before the reaction slows down. Central experiments are the most effective based on the speed of the process. The results show that **when there is 100.63mg/L of H_2O_2 and at least 4mg/L Fe(II), over 80% of mineralization rate is achieved** (Figure 17).

Increasing the Fe(II) up to 7.5 mg/L, the speed of the reaction rises and the mineralization rate is slightly higher. Furthermore, the concentration of 11.04 mg/L is not anymore improving the results, but decreasing them instead. The best concentration of the Fe(II) could be found between 3.96 mg/L and 7.5 mg/L or slightly above the latter. Most likely the best concentration is quite close to 7.5 mg/L, but it could be assured by further studies.

Experiments C(161_5) and D(161_10) are second best regarding the speed. Experiment I(100.63_11.04) is also fast, but as noted earlier, increasing the amount of Fe(II) that much will reduce the mineralization rate.

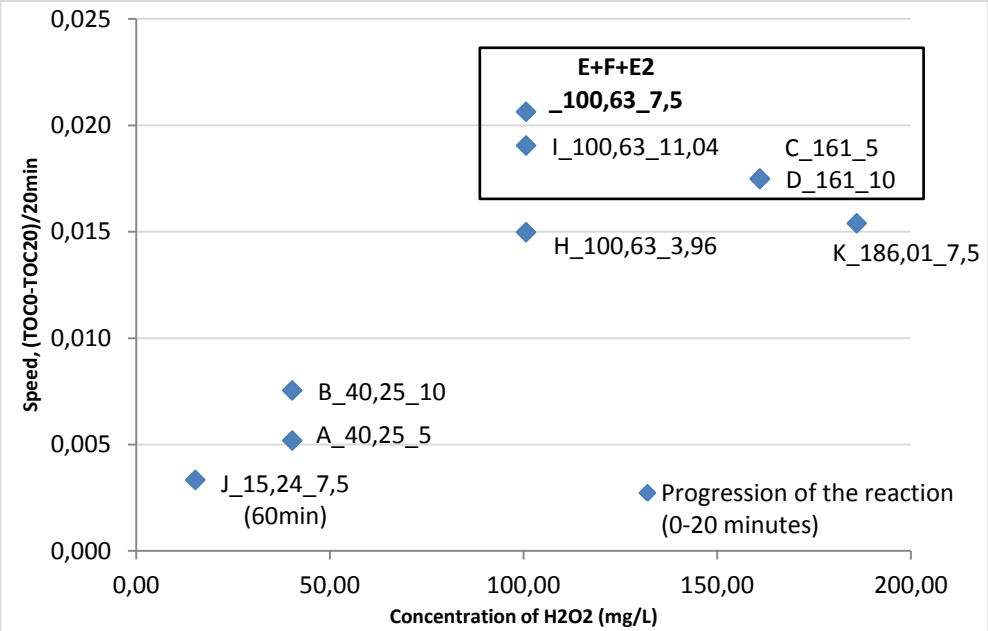


Figure 18. The best results according to the speed of the reaction. Calculation is based on the speed during first 20 minutes of the photo-Fenton process. SD was less than 0,03 in all experiments B, D, and E+F+E2, the others did not have replicates.

In Navarro's study, the concentrations of 80.5 mg/L, 104.08 mg/L, 120.75 mg/L, 161 mg/L and 201.25 mg/L of H_2O_2 were studied. In her study, the stoichiometric amount of H_2O_2 was considered the best. Comparing the results of the experiments having fewer H_2O_2, there can be seen some variation. There were 3-4 experiments in each category. It was quite clear that 80,5 mg/L was not enough and after 90 minutes only 60% of by-

products were mineralized. Using 104.08mg/L, 60-75% was mineralized, with 120.75 mg/L 65-80% was mineralized and with 161 mg/L close or over to 80% (amount of Fe(II) also varies). (Navarro 2013, Appendix 3, 53-61.)

However, this study showed clearly that the concentration of 100.63mg/L was even better than 161 mg/L, which was not consistent with the previous study. It should be noted that this study also included some variation (from 65% to over 80% mineralization after 90 minutes) with central experiments (100.63_7.5), but one experiment was excluded because of the adjusted temperature. After the excluding, the mineralization was clearly over 80%. Overall the variance can be caused from various reasons, including the temperature and other conditions as well as human error. Because there was slight variance in both studies, more studies around the concentrations of 100.63-161 mg/L Fe(II) would give more reassurance about accuracy of the results of this study.

5.2 BOD_5 of the experiments

5.2.1 Control samples

Control samples were analysed in order to evaluate the reliability of the measurement as well as define the BOD_5 value of the blank sample to subtract it from the BOD_5 values of the samples. The dissolved oxygen was measured with DO-meter and BOD_5 with respirometric Oximeter.

The DO depletion values of control samples were 0.13 and 0.07 mg/L, when the mean value is 0.10 mg/L (Appendix 7, Table 3). This is less than 0.2 mg/L, which was the limit according to the instructions. In Table 14, the actual BOD_5 values of the control samples are presented. The mean value (7 mg/L) is used to correct the BOD values of all samples (Appendix 11).

Since the blank controls were fine and seed controls close to requirements, it was evaluated that BOD results are reliable enough to make conclusions regarding this study (Appendix 7). After all, the BOD analyses have many variables and in this study the main purpose was to get the basic idea about the biodegradability of BPA and intermediates, so the method was considered reliable for this purpose.

Table 14. BOD_5 of the control samples, one sample in each equipment. The mean value was taken off from all the sample values.

BOD_5 of the control:	Eq 1	Eq 2	Eq 3	Eq 4 (prelim. test, higher temp.)	Mean
	7	**7**	**5**	**9**	**7**

5.2.2 The biodegradability of the experiments

BOD_5 and BOD_7 were measured from the end solutions of the photo-Fenton experiments. The data is presented in Appendix 11. Based on the results, all solutions were at least partially biodegradable after the photo-

Fenton process (Table 15). In Figure 19, it can be seen that based on the DOE, which included only the first experiments (blue bars) and 3 central values, the results seem quite reliable and the variation between three central values is minor. Final four (H-K) experiments were not performed due to the limited resources.

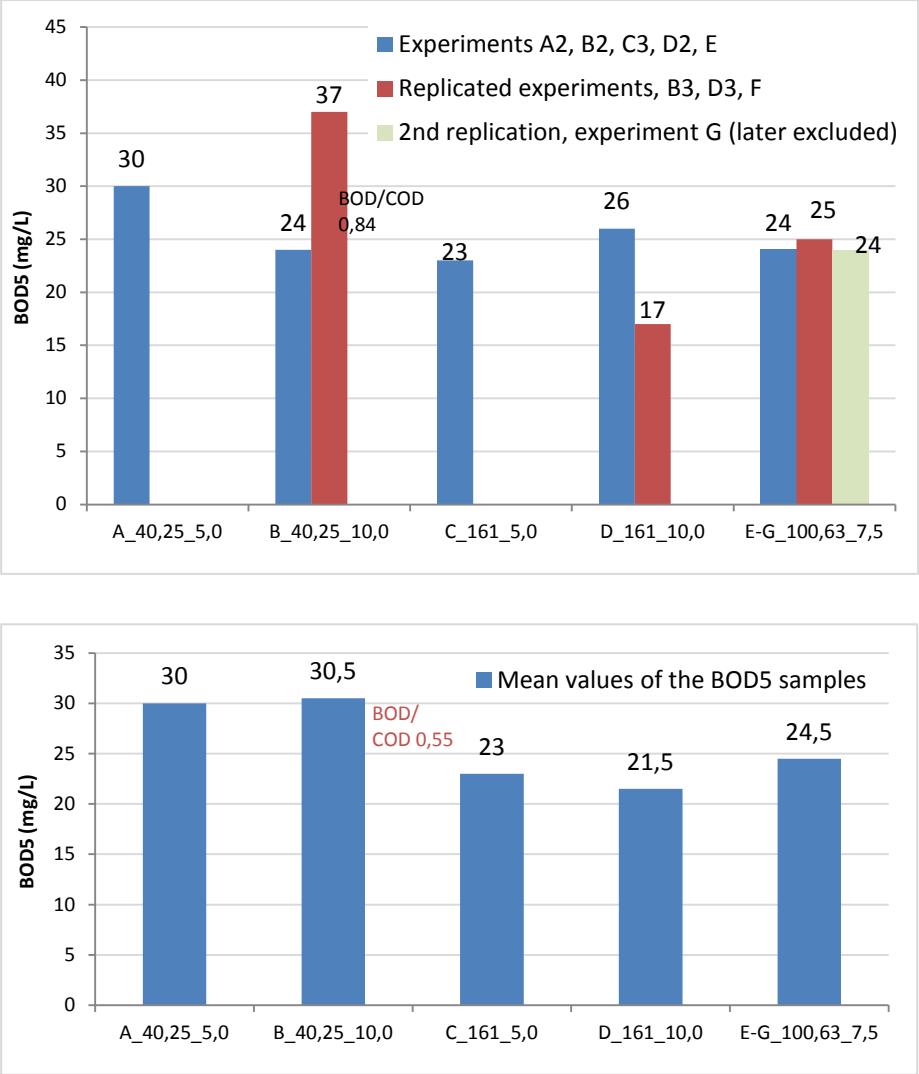


Figure 19. BOD_5 of the end solutions of the experiment (blue) and the replicates (red). The mean BOD_5 values are presented in the lower graph. When using the mean values in the further analysis of the results, the variation of the results should be kept in mind. (Because experiment G was excluded later, the mean value of centrals (24.5) is only from experiments E and F.)

After all, it was decided to do two replicates of the samples B and D (Figure 19, red bars), which unfortunately showed quite significant variation between the samples. This variation should be noted, if the mean values are used in further analysis. As seen in the graph above, the

central experiments (E-G) were quite similar, but the variation of experiments B and D was significant. The TOC results (Appendix 12) were very similar in these replicates, so they do not explain the difference in BOD. The experiment B had also a very high TOC value and D quite low, since it is close to the optimum concentration of reagents.

To improve accuracy, it would have been better to have replicates of all samples. Furthermore, the variation still does not have effect on the evaluation of biodegradability in most of the cases (C, D and the centrals). Experiment B is biodegradable if using the mean value, but using only the first lower value (24 mg/L), B is only partially biodegradable. Experiment A could have also given the same result, if there had been similar variation.

But since in these experiments (A and B) the amount of H_2O_2 was very small, and they were not even close to best ones considering the effectiveness of the photo-Fenton process, it can be concluded that variation does not have remarkable effect on results. Regardless the possible variation, the biodegradability of the solutions C, D and E+F is clear, and A and B are also at least partially biodegradable. As a conclusion, for the purpose of this study the results are reliable enough. Furthermore, the results show that when H_2O_2 concentration is over 100.63mg/L and there is at least 4 mg/L Fe(II), the end solution after the photo-Fenton process is biodegradable. If there is only 40.25 mg/L of H_2O_2, the BPA is degraded, but TOC is high and there still exist by-products that may effect on total biodegradability.

Table 15. Biodegradability of the end solutions of the experiments. B and D are calculated using the mean value of two measurements. Biodegradability is based on BOD/COD ratio: biodegradable > 0.6, partially biodegradable 0.4-0.6 and not biodegradable < 0.4. Reliability of A & B should be noticed (Figure 19).

Experiment	TOC (mg/L)	COD (mg/L)	BOD5	BOD5/ COD	biodegradability
A2_40.25_5.0	17.16	45.77	30	0.66	Yes (partially?)
B(2,3)_40.25_10.0	16.443	43.85	30.5	0.70	Yes (partially)
C(2,3)_161_5.0	3.4065	9.09	23	2.53	yes
D(2,4)_161_10.0	3.349	8.93	21.5	2.41	yes
E-G_100.63_7.5	3.122	8.33	24.5	2.94	yes

5.3 The biodegradability of the BPA solutions

Table 16 presents the biodegradability calculations based on theoretical COD value and BOD results. The BPA solution of 30 mg/L is not biodegradable, the result being consistent with Navarro's results. In addition it was found out that BPA solution of 20mg/L is partially biodegradable and 10 mg/L and less are totally biodegradable. Biodegradability of BPA in different concentrations is presented in Figures 20 and 21.

In Navarro's study, BPA solution of 10 mg/L was only partially biodegradable. Because the concentration of the aliment was higher in this study, and therefore the method more reliable, the result of this study could be considered more reliable in this case.

Table 16. Biodegradability of the BPA solutions. Biodegradability is based on BOD/COD ratio: biodegradable > 0.6, partially biodegradable 0.4-0.6 and not biodegradable < 0.4.

BPA (mg/L)	TOC (mg/L)	COD (mg/L)	BOD5	BOD5/COD	**Biodegrada-bility**	Biodegradability, Navarro's study
0	0.001	0.003	34	12748.4	**yes**	yes
2.5	1.85	4.938	24	4.86	**yes**	yes
5	3.84	10.244	23.5	2.29	**yes**	yes
7.5	5.64	15.047	20	1.33	**yes**	-
10	7.54	20.100	17.5	0.87	**yes**	partially
20	15.69	4.,832	23	0.55	**partially**	-
30	24.43	65.165	22.5	0.35	**no**	no

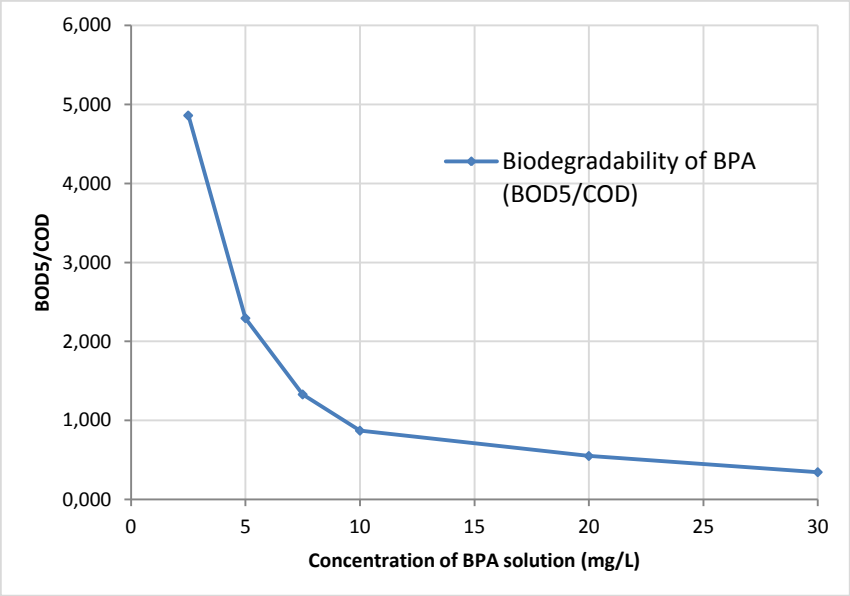


Figure 20. The calibration curve indicating the biodegradability of BPA in different concentrations. The graph shows the BOD_5/COD relations of the BPA samples.

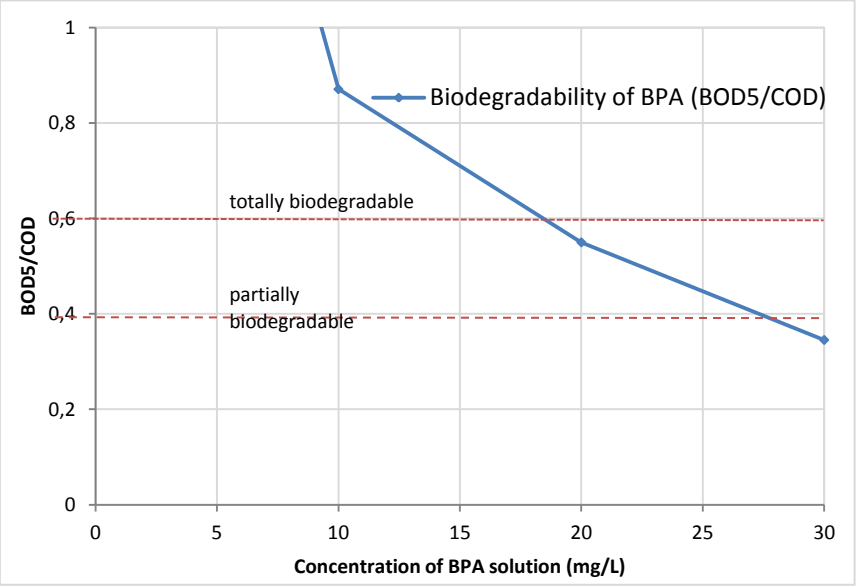


Figure 21. The biodegradability of BPA solutions. Based on the curve, the concentrations less than 18 mg/L BPA are totally biodegradable, about [18-27 mg/L] are only partially and above that are not biodegradable.

5.4 The comparison of BOD_5 and BOD_7

Determination of BOD_5 was the main idea of this study in order to get results about the biodegradability of BPA and the end solutions of the experiments. However, the BOD_7 was also recorded for curiosity due to a fact that it is used in some countries as a parameter of the quality of the water.

It was found out that the difference between BOD_5 and BOD_7 was not significant. The BOD_7 values were quite consistently slightly higher than BOD_5 values (Figure 22). In lower concentrations, the difference was a bit higher than in higher concentrations of BPA. Is should be also noted that for technical and practical reasons, the value of 360 mL samples was BOD_8 instead of BOD_7 and for this reason probably slightly higher than the BOD_7 of 480 mL samples (Appendix 6). If the variation of two highest BOD_8 results (2.5 and 5 mg/L) is ignored, the overall difference in BOD_5

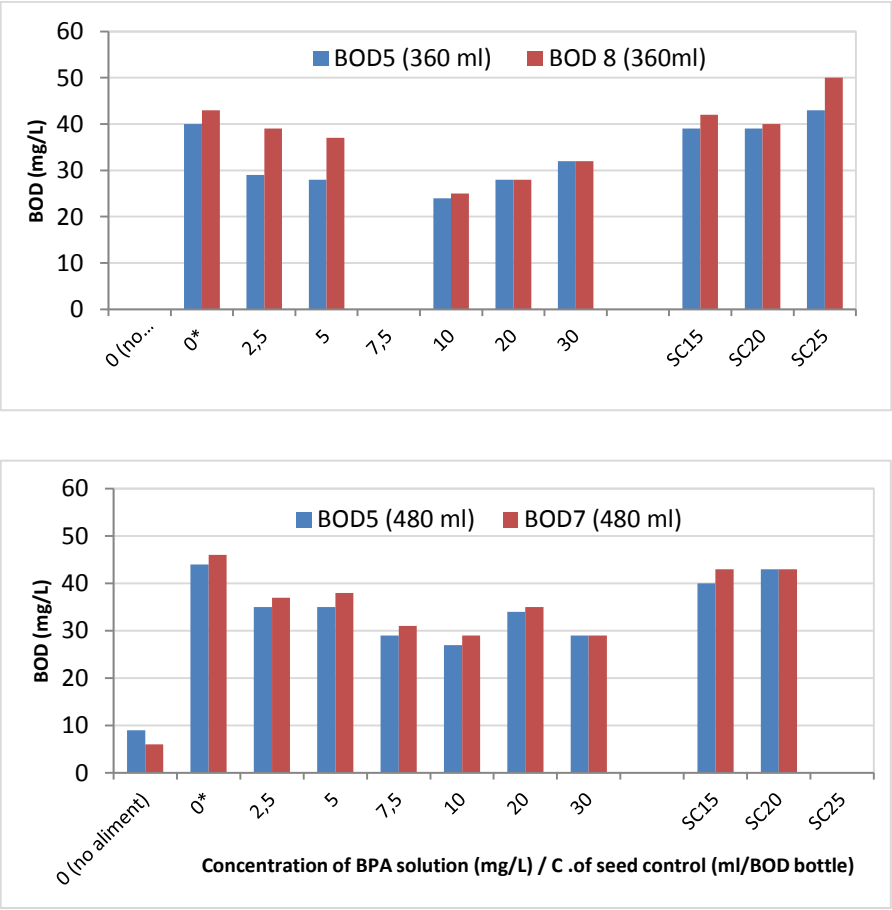


Figure 22. The difference in BOD_5 and BOD_7 values. For practical and technical reasons for sample sizes of 360 mL the recorded value was BOD_8 instead of BOD_7.

and $BOD_{7(8)}$ results was less than 3 mg/L. Especially the 480 mL samples show quite clearly that BOD_7 is systematically 2-3 mg/L higher than BOD_5.

The end-solutions of the experiments show similar results, which are presented in Figure 23. In all cases the difference between BOD_5 and BOD_7 was less than 2 mg/L.

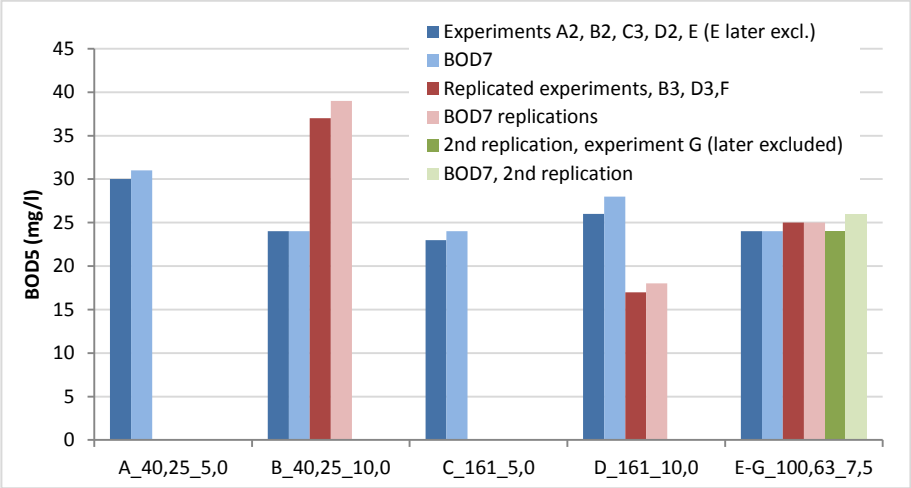


Figure 23. BOD_5 and BOD_7 of the experiments. As expected, BOD_7 (lighter colours) was slightly higher than BOD_5. Variation between the replications (samples B and D) is discussed in Figure 19.

5.4.1 The effects of different amounts of the aliment on the BOD test

Unfortunately the time and resources were limited, and in addition there were technical problems with the BOD equipment. Therefore, there were not enough results to evaluate the effects of the different amounts of aliment on the BOD test.

5.5 Toxicity

Toxicity was analysed using available two bacteria, *Escherichia coli* and *Staphylococcus epidermidis.* The method was not the best for analysing the toxicity of BPA, but the aim was also to learn the technique. In further studies of toxicity of BPA, some other method presented in Chapter 2.5.2 could be more successful.

After reading the growth of the bacteria, the results were normalized in relation to maximum growth (control samples without contaminant). The mean value of three samples was used, so the standard deviation is based on those three values. The summary of the results is presented in Appendix 13.

5.5.1 The effects of BPA on available bacteria

The results seem to indicate that BPA as well as intermediates were not toxic for the available bacteria. Toxicity was not detected in such low concentrations of BPA used in this study (30 mg/L).

Toxicity of BPA solutions of different concentrations was analysed using two sets of solutions in order to get more accurate results. As expected, the toxicity results show that BPA was not toxic for the either bacteria in such low concentrations, but instead it was used as aliment for *S. epidermidis* (Figure 24). This result is in line with the research results presented in Chapters 2.1.3 and 2.1.4.

As seen the graph above, particularly *S. epidermidis* seems to use BPA as aliment, especially when the concentration is higher than 10 mg/L. The results with two different solutions are quite similar, unlike the results of *E. coli,* which have more variation. Results of *E. coli* in low concentrations are not consistent.

Graphs also show that nearly all the results fit in the standard deviation limits of the control samples. The interpretation could be that results of all the BPA solutions are quite equal to the maximum growth without contaminant (apart from last two of *S. epidermidis)*.

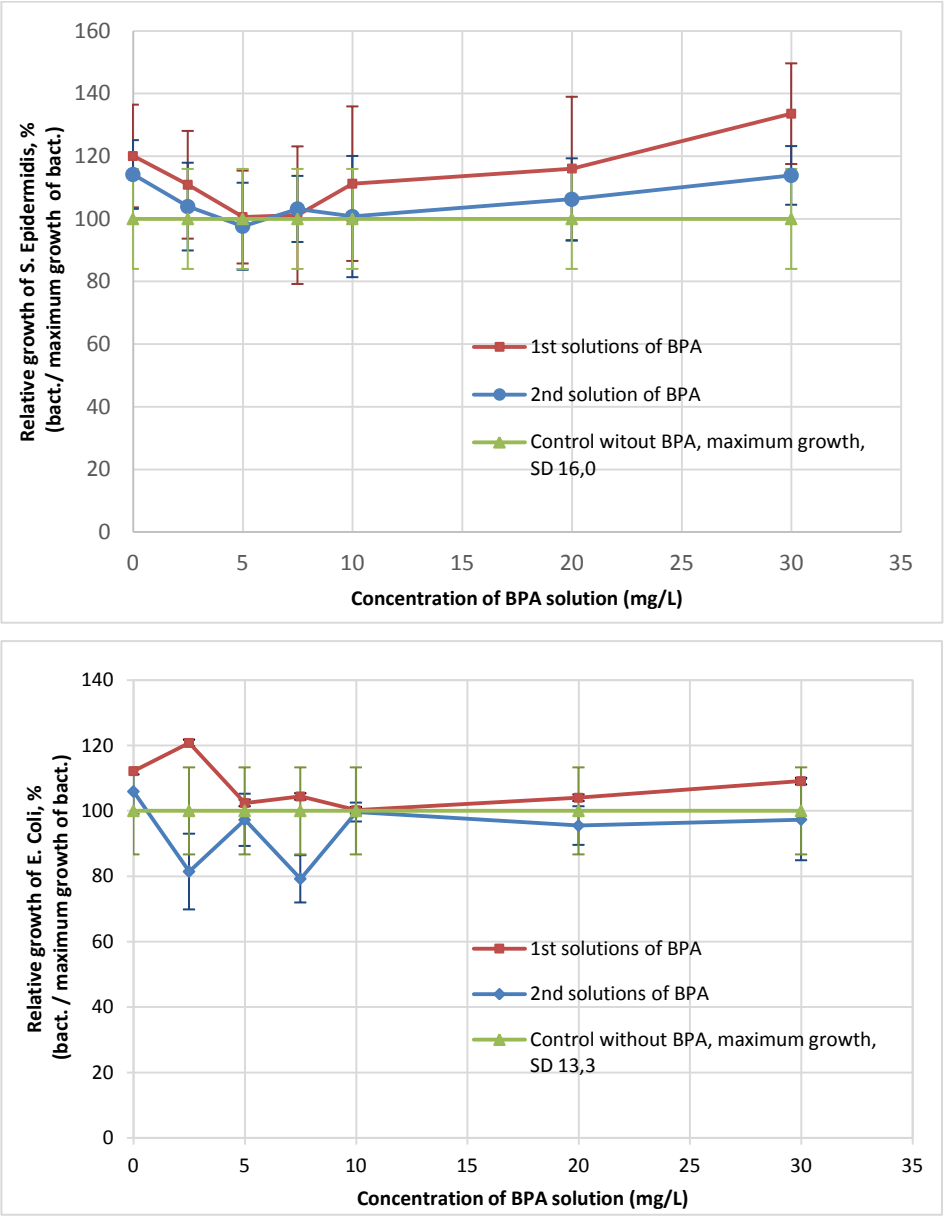


Figure 24. Growth of the bacteria on BPA solutions. Concentration of BPA was too low to study toxicity. The results of *S. epidermidis* had the standard deviation of 9.4-24.7 and *E. coli* 2.9-12.4.

5.5.2 The LD_{50} of BPA

The results of the toxicity analyses seemed to indicate, that low concentrations of BPA or by-products did not have effect on bacteria. The LD_{50} value was determined in order to get reassurance about the toxic concentration level for the used bacteria. LD_{50} value is dependent on the bacteria or other organism used. It should be noted, that the determined LD_{50} value is not directly comparable to some other strain of bacteria.

Figure 25 presents that the LD_{50} doses for both bacteria are very high, approximately 40000 µg/mL, which is equivalent to mg/L. This result confirms that tracking the toxicity of BPA solutions of this study in available bacteria is infeasible. The concentration of BPA solution should be so high that it is not appropriate regarding the purpose of this study or usual wastewater concentrations needing treatment.

However, because the available method was to use these bacteria, they were used in order to learn the technique.

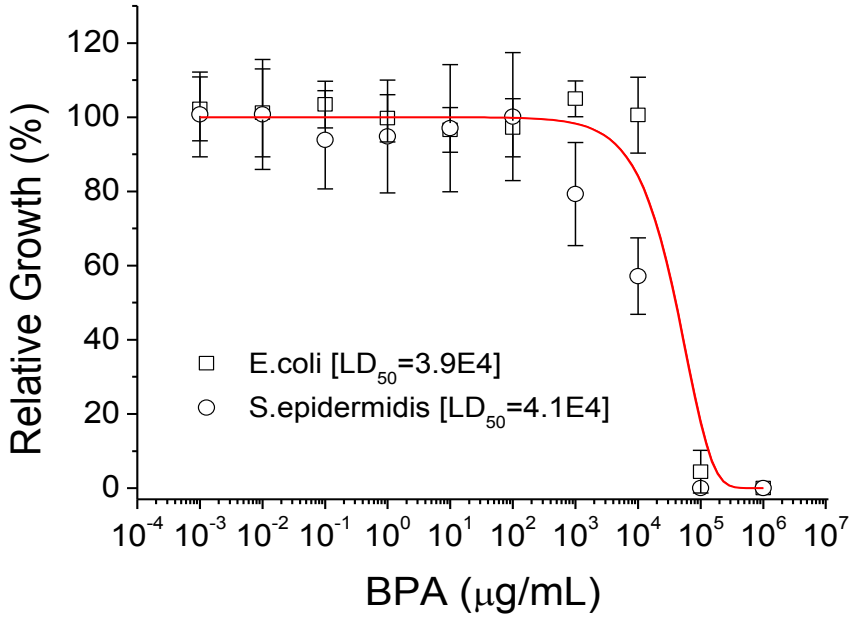


Figure 25. LD_{50} of BPA. The LD_{50} doses of BPA for both bacteria are very high (approximately 40000 ppm).

5.5.3 The effects of the remaining H_2O_2 on the bacteria

According to the Pérez-Moya et al. (2007), remaining H_2O_2 effects on the bacteria and limits the growth. This effect could be seen also in this study and the experiment C2 is presented as an example (Figure 26). Both bacteria are affected and not growing when there is more than 70% of H_2O_2 left. After this point, *E. coli* begins to grow rapidly, but *S. epidermidis* is more sensitive and is not growing until there is less than 15 % of H_2O_2 left.

As comparison, Figure 27 presents the experiments that have only small amount of H_2O_2 in the beginning. In most cases, all of the H_2O_2 or at least 80% was consumed already after 10 minutes. These experiments show that the growth of the bacteria is more stabile, and it can be assumed that the variation is caused by by-products. It should be noted that for the discussion of the effects or toxicity of the by-products, only the results containing less than 20% of H_2O_2 should be included. To make sure H_2O_2 is not effecting, it means only samples after 40 minutes (see Figure 13), although the time varies depending on the experiment and the amount of the reagents. However, in this case the toxicity was not under discussion due to the low concentration of contaminant.

E. coli stay close to the maximum growth, but *S. epidermidis* seems to be able to use BPA as well as by-products as aliment, because the growth is above the maximum growth, also in the beginning when there exists H_2O_2 (Figures 26 and 27). Similar behaviour of *S. epidermidis* was detected also when BPA solutions were tested (Figure 24).

Microbiological analyses always have uncertainty and their accuracy is highly dependent on the thorough working method. Contamination can easily cause inaccuracy. Previous graphs also show that standard deviation varied a lot being very small (0.03) in some cases but as high as 0.59 in the worst case. Results need to be analysed carefully and excluded the ones most likely having an error.

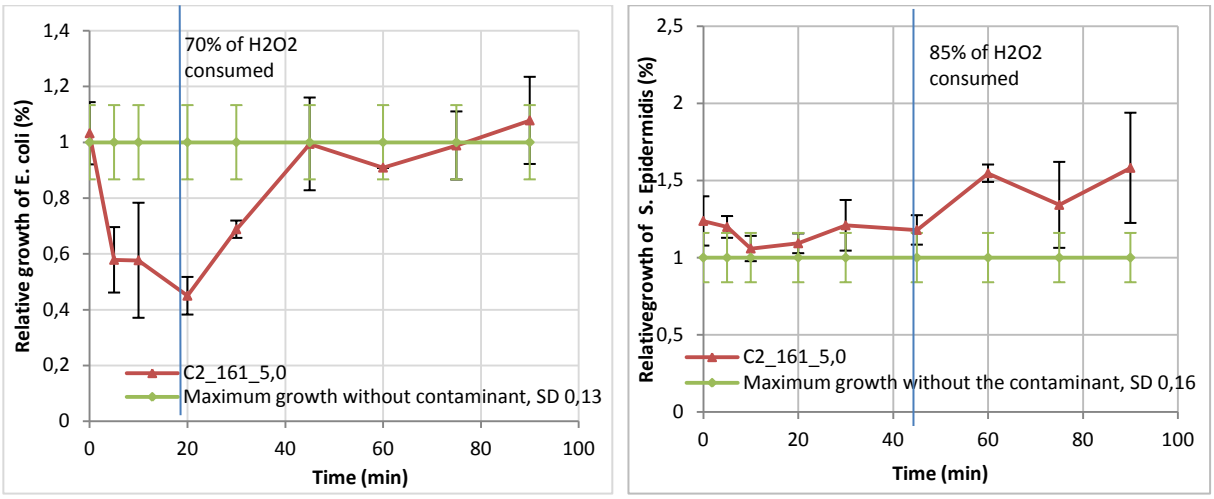


Figure 26. Effect of H_2O_2 for the bacteria is clearly visible in the graphs. Approximately 70% of H_2O_2 was consumed after 20 minutes and 85% after 45 minutes. *S. epidermidis* seems to be more sensitive for existing H_2O_2 than *E. coli.* It should be noted that 0-value is the value of BPA [30mg/L] without H_2O_2, because samples were not taken in the beginning of the experiment (the first sample was taken after 5 minutes).

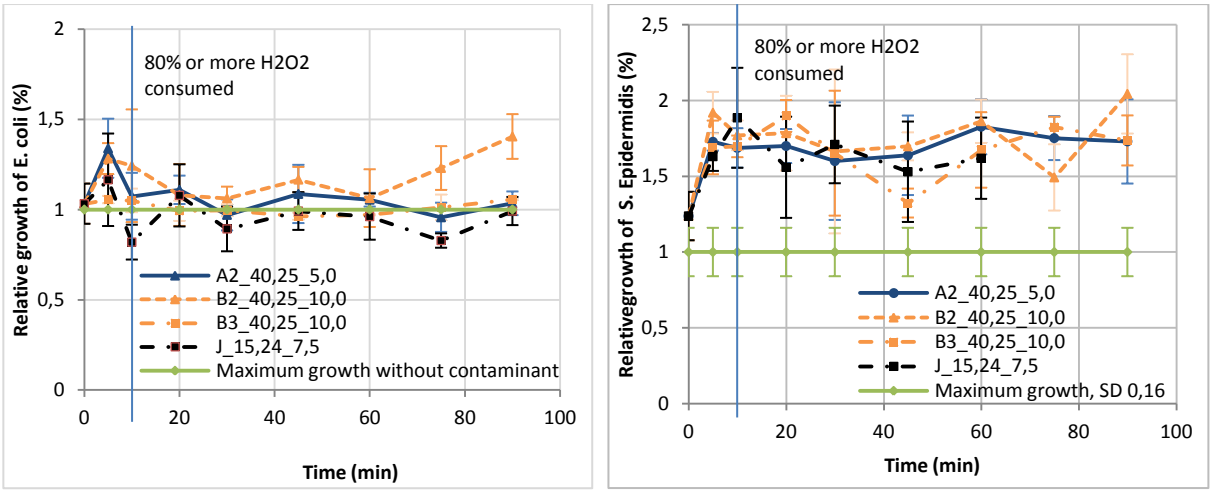


Figure 27. Experiments containing very little H_2O_2 were more stable than C2 (Figure 26). In most cases all of the H_2O_2 was consumed already after 10 minutes. In this case *S. epidermidis* is also growing above the maximum growth level, which indicates that it can use BPA and by-products as aliment.

Clear consistency among the rest of the results was not detected, because the BPA concentration was too low to show clearly possible toxic effects of the by-products. The summary of the results is presented in Appendix 13.

6 CONCLUSIONS

6.1 Conclusions

The goals of this study were achieved (Chapter 1.3), especially considering the time available for the project. Nearly all the research questions were answered, even though some uncertainty was left and further study needed. Especially, the whole project was very instructive for the author in many ways.

In accordance to previous studies, photo-Fenton reaction was found to be suitable process to eliminate BPA and its by-products. TOC concentration was decreased significantly during several 90 minute experiments. Navarro's previous study (2013) indicated that BPA disappeared in a few minutes, and after that by-products appear until the mineralization has reached the stabile state. In this study, only the experiments containing small amount of H_2O_2 (A and B, 40,24 mg/L), did not lead into the high mineralization rate. In all other experiments, close to 80% was mineralized.

H_2O_2 concentration

The amount of H_2O_2 mainly affects the costs of the photo-Fenton process, because it is the main reagent. Fe(II) can be considered as catalyst. Navarro concluded that stoichiometric amount of H_2O_2 (161 mg/L) was close to the best concentration. In this study, it was found out that 161 mg/L is not necessarily needed, and 100.63mg/L is enough to achieve almost the same mineralization rate.

In conclusion, the results suggest that when there is 100.63mg/L of H_2O_2 and at least 4mg/L Fe(II), over 80% of mineralization rate is achieved. Because the concentrations of both reagents were the lowest of this study (A and B not included), but still achieving the good mineralization rate, it can be concluded that both research questions (2 i and 2 ii) have the same answer. BPA and the by-products can be removed using these

concentrations (lowest if A and B are not included), and simultaneously they are the ones giving best mineralization rate.

In this study, the DOE did not consist experiments between 40.25 mg/L and 100.63 mg/L. According to Navarro (2013), the concentration of 80,5 mg/L of H_2O_2 was not enough, because after 90 minutes only 50-60% of by-products were mineralized. Compared to the Navarro's study, in this study all the results consistently indicated that lower concentrations of reagents are sufficient (Table 17). Therefore, it may be possible that less than 100.63mg/L of H_2O_2 is enough to remove the BPA, depending on the level of mineralization wanted. It is also possible that either of the studies has some inaccuracy for some reason. However, based on this study, the best mineralization rate seems to be close to 100.63mg/L. Whether the concentration of H_2O_2 could be even lower or not, could be studied in the future.

Table 17. Approximate mineralization after 90 minutes experiment (amount of Fe(II) varies). Comparison of the results of this study and Navarro's results.

	H_2O_2 40.25 mg/L	**H_2O_2 80.5 mg/L**	**H_2O_2 100.63 mg/L**	**H_2O_2 104.08 mg/L**	**H_2O_2 120.75 mg/L**	**H_2O_2 161 mg/L**
Navarro (2013)		50-60%		60-75%	65-80%	80-85%
The results	30-35%		80-85%			80-85%

In this study, there was no time to confirm the results with replicates. Further studies could be performed around the concentrations from 80.5 mg/L to 100.63 mg/L of H_2O_2 to find the best amount considering the effectivity and cost-efficiency of the process. Due to the lack of replicates in this study, some experiments of higher concentration than 100.63 mg/L, could be also included.

Fe(II) concentration

If the concentration of Fe(II) was increased up to 7.5 mg/L (100.63 mg/L H_2O_2), the speed of the reaction rose and the mineralization rate was

slightly higher. Furthermore, the concentration of 11.04 mg/L of Fe(II) did not anymore improve the results, but decreased them instead. The best concentration of the Fe(II) could be found between 3.96 mg/L and 7.50 mg/L or slightly above the latter. Most likely the best concentration is quite close to 7.5 mg/L, but it could be assured by further studies.

Biodegradability

BOD tests showed that BPA concentration 30 mg/L was not biodegradable, 20 mg/L was partially biodegradable and the lower concentrations were totally biodegradable.

Based on the results, after the photo-Fenton treatment, all the solutions were at least partially biodegradable. The experiments C(161_5), D(161_10) and E+F+E2(100.63_7.5) were totally biodegradable. These results were slightly different than Navarro's, who studied also the effects of different concentrations of aliment. In conclusion, the chosen amount of the aliment in this study appeared to be suitable for the rough estimation of biodegradability. These results filled the gaps and the uncertainty Navarro had on her results.

Toxicity

It was reassured that in such low concentrations, BPA neither the by-products are toxic for the available bacteria (*S. epidermidis and E. coli*). Instead it seems that bacteria, especially *S. epidermidis* could use BPA as well as by-products as an aliment source. This result is in line with the previous research about biodegradability of BPA and biodegradation as a BPA removal technique (Chapters 2.1.4 and 2.1.5).

LD_{50} for BPA was determined for both bacteria. A lethal dose was very high (40000mg/L), confirming that tracking the toxicity of BPA and by-products using available bacteria was infeasible in such low concentration.

6.2 Other observations

It was also found out that the overall difference in BOD_5 and $BOD_{7(8)}$ results was quite small, but systematically $BOD_{7(8)}$ was 2-3 mg/L higher than BOD_5. It should also be kept in mind that BPA is classified as ready biodegradable using 28-day test (Chapter 2.1.2).

Based on the results, the remaining H_2O_2 was clearly affecting the bacteria as Pérez-Moya et al. (2007) also had noted. Both bacteria were affected and did not grow when there was more than 70% of H_2O_2 left. After this point, *E. coli* began to grow rapidly, but *S. epidermidis* was more sensitive and was not growing until there was less than 15 % of H_2O_2 left. For the discussion of the effects or toxicity of the by-products, only the results containing less than 20% of H_2O_2 should be included. However, in this case, the toxicity was not under discussion due to the unsuitable method.

As an academic study, this project provided the opportunity to learn different techniques related to wastewater quality analysis and AOPs. Toxicity was tested using an available method, but to get real results in the case of BPA, the suitable method would have needed more background research. Some research was found after the practical part to assure that biodegradation has been studied as available method for BPA removal. Based on this information, it could have been possible to infer that bacterial methods are not suitable for BPA. For further toxicity studies, other methods (Chapter 2.5.2) should be considered.

As Rizzo (2011) also mentioned, extra cautiousness should be remembered when comparing biodegradability and bacterial toxicity in case of AOPs, because the effects of the pollutants vary depending on the bacterial strain (Chapter 2.5.2). It is possible that the effects on biodegradability or toxicity will be under- or overestimated, because the pollutant, such as BPA, may have harmful affect on one bacteria strain but no effects on other strains.

Because bacteria commonly employed in the biological treatment stage have no effect on persistent substances (Fraunhofer 2014), one option

could be to ensure that mainly all the TOC is eliminated and then toxicity assays would not be needed. However, the combination of AOPs and biodegradation most likely will be studied more in the future as one possibility to remove pollutants, such as BPA, from the wastewater. The results of the further studies may also influence on the duration and the qualities of used AOP, if the bacteria can take care of the certain amount of remaining BPA.

6.3 Reliability of the study and the methods

There are many factors affecting the reliability of the BOD test, for example the cleanliness of the equipment, the freshness of the aliment solution, a correctly prepared seed solution, and a proper way of handling the samples and the bottles. The preliminary tests showed that there is no big difference if using the sample size of 360 mL or 428 mL and the first one was chosen for the study.

Overall, the BOD method was considered reliable enough for the purpose of the study, although there was some variation among the replicates. The variation was considered to have a minor effect, because the possible effect on the results included only experiments A and B (partially/totally biodegradable) that were not relevant in order to find the best results. If more resources were available, replicates of all samples could have increased accuracy of the method. After all, the variation does not change the conclusion that experiments C, D and centrals (E+F+E2) were totally biodegradable after the photo-Fenton process.

The amount of the aliment (50 ppm) seems to have been a good choice based on the results. If there had been more time, it could have been possible to run the tests also with other amounts of the aliment, for example half (25 ppm) and double aliment (100 ppm). Only a couple of these samples were carried out, but because of technical problems and limited time there were not enough results to make conclusions.

The reliability of the experimental method can also be questioned. There are number of factors affecting the photo-Fenton process, as well as the analytical methods. Human error is always possible and especially even a small error when measuring the reagents could have affected, because the quantities of the reagents were very small. Even though sampling was implemented systematically the same way, it is possible to have an effect on results if there's difference in timing or handling the samples. Samples were kept in ice and the toxicity samples were frozen as soon as possible, but still the conditions may have varied. The TOC analyzer was slow and it was not possible to analyze samples immediately. Previously in EUETIB, the samples had been tested, and it was found out that the reaction stopped when the sample was kept cold. Anyway, in some cases of this study the last samples were kept in ice over an hour, which may have caused inaccuracy.

Conditions of the experiment were set as stable and repeatable as possible and the effects of pH and temperature were monitored. It was difficult to keep the temperature stable, because the light was heating the container. It was noted that letting the temperature slightly rise towards the end of the experiment, had only minor effect on the process and was considered acceptable. Cooling down the process was difficult to control manually and to keep the temperature at +25ºC was interfering the process and affecting the results. It was chosen not to interfere the temperature, but to try to keep it stable in the beginning in order to get as repeatable conditions as possible. The experiments of this study had quite similar conditions, and it was considered that the temperature did not have effect on the results. However, when comparing the results for example to Navarro's results, it is possible that slightly higher temperature has lead to slightly better results in some cases.

It would be interesting to study more about the effects of the temperature to the photo-Fenton process and mineralization rate of BPA. This study raises the question, if the slightly higher temperature (+25 +2ºC) together with the certain amount of the reagents affected in a way that the concentration of 100.63mg/L of H_2O_2 gave better results than 161 mg/L

Navarro mentioned. Overall, anyway in this study, it was noted that amount of H_2O_2 is more important than the amount of iron.

Based on the TOC results, the reliability of the experimental method can be considered good. Standard deviation of the TOC of the replicate samples (90 min) was less than 0.06 in all cases there were replicates. However, more replicates would have increased reliability. As discussed in Chapter 5.1.1, the usage of several solutions and dilutions did not either have effect on the results.

6.4 Improvements and recommendations for further studies

Some ideas for the improvements and further studies are collected here:

- Further studies could be performed around the concentrations from 80.5 mg/L to 100.63 mg/L of H_2O_2 to find the best amount considering the effectivity and cost-efficiency of the process.
- Effects of the temperature during the photo-Fenton on the mineralization rate of BPA.
- Further study of biodegradability during the photo-Fenton reaction. Since the end solution of all experiments was biodegradable, it could be determined at what point of the reaction the solution is biodegradable. Based on the biodegradability and TOC results of this study, it could be created a model that compares the TOC during the experiment and indicates when the biodegradable level has been achieved. The length of the process could be minimized, even though some TOC is left.
- When studying BOD, enough replications would help to evaluate the accuracy, accuracy could also be improved comparing the different amounts of aliment used.
- Since eliminating mainly all the TOC would be enough to achieve biodegradability and since the end solution is not toxic, there should not be a need for further toxicity assays. But if one would study toxicity of the BPA, the suitable method (e.g. invertebrates, fish, sea urchin, mammal cells) should be found.

- AOPs, such as the photo-Fenton and biodegradation as combined treatment method, would be interesting research area.
- If the use of BPA will be restricted e.g. in thermal paper production or other industry in the EU, the use of BPA will decrease remarkably. Therefore also the substitutes, such as BPS (bisphenol-S), could be studied.

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Grandparents Tuula and Perttu, as well as my dear sister Riia have helped with the kids and made it possible for me to work also during the day time - I hope you all know that you have been indispensable.

Thanks to all who have helped me in any way.

Safety Information of BPA, ICSC: 0634

BISPHENOL A	**ICSC:** 0634 Peer-Review Status: 09.06.2011 Validated

4,4'-(1-Methylethylidene)bisphenol
4,4'-Isopropylidenediphenol

CAS #: 80-05-7 RTECS #: SL6300000
EC #: 604-030-00-0
EINECS #: 201-245-8

Formula: $C_{15}H_{16}O_2$ / $(CH_3)_2C(C_6H_4OH)_2$
Molecular mass: 228.3

TYPES OF HAZARD / EXPOSURE	ACUTE HAZARDS / SYMPTOMS	PREVENTION	FIRST AID / FIRE-FIGHTING
FIRE	Combustible.	NO open flames.	Use water spray, foam, powder, carbon dioxide.
EXPLOSION	Finely dispersed particles form explosive mixtures in air.	Closed system, ventilation, explosion-proof electrical equipment and lighting. Prevent deposition of dust.	In case of fire: keep drums, etc., cool by spraying with water.
EXPOSURE	**See EFFECTS OF LONG-TERM OR REPEATED EXPOSURE.**	**PREVENT DISPERSION OF DUST! AVOID ALL CONTACT!**	
Inhalation	Cough. Sore throat.	Use local exhaust or breathing protection.	Fresh air, rest. Seek medical attention if you feel unwell.
Skin	Redness.	Protective gloves. Protective clothing.	Remove contaminated clothes. Rinse and then wash skin with water and soap. Seek medical attention if you feel unwell.
Eyes	Redness. Pain.	Wear safety goggles or face shield.	First rinse with plenty of water for several minutes (remove contact lenses if easily possible), then refer for medical attention.
Ingestion	Nausea.	Do not eat, drink, or smoke during work.	Rinse mouth. Give one or two glasses of water to drink. Refer for medical attention .

SPILLAGE DISPOSAL	PACKAGING & LABELLING
Personal protection: particulate filter respirator adapted to the airborne concentration of the substance. Do NOT let this chemical enter the environment. Sweep spilled substance into sealable containers. If appropriate, moisten first to prevent dusting. Carefully collect remainder. Then store and dispose of according to local regulations.	**EC Classification** Symbol: Xn; R: 37-41-43-52-62; S: (2)-26-36/37-39-46-61 **UN Classification** **GHS Classification** Signal: Warning Causes serious eye irritation May cause an allergic skin reaction Suspected of damaging fertility or the unborn child May cause respiratory irritation Toxic to aquatic life 

EMERGENCY RESPONSE	SAFE STORAGE
	Separated from acid anhydrides, acid chlorides, strong oxidants, strong bases and food and feedstuffs. Store in an area without drain or sewer access.

IMPORTANT DATA

Physical State; Appearance
WHITE CRYSTALS FLAKES OR POWDER.

Physical dangers
Dust explosion possible if in powder or granular form, mixed with air.

Chemical dangers
Reacts violently with strong oxidants. This generates fire and explosion hazard. Reacts vigorously with acid anhydrides, acid chlorides and strong bases. This generates heat and pressure-rise explosion hazard.

Occupational exposure limits
TLV (NOT-ESTABLISHED):.
MAK (inhalable fraction): 5 mg/m³; Peak limitation category: I(1); Pregnancy risk group: C; Photosentization (SP); BAT issued; (DFG 2010).
EU OEL (inhalable fraction selected): 10mg/m³ as TWA;.

Routes of exposure
The substance can be absorbed into the body by inhalation of its aerosol.

Inhalation risk
Evaporation at 20°C is negligible; a nuisance-causing concentration of airborne particles can, however, be reached quickly when dispersed, especially if powdered.

Effects of short-term exposure
The substance is severely irritating to the eyes. The substance is mildly irritating to the respiratory tract.

Effects of long-term or repeated exposure
Repeated or prolonged contact may cause skin sensitization and photosensitization. The substance may have effects on the upper respiratory tract. Ingestion may cause effects on the liver and kidneys. Animal tests show that this substance possibly causes toxic effects upon human reproduction.

PHYSICAL PROPERTIES	ENVIRONMENTAL DATA
Boiling point at 1.7kPa: 250-252°C Melting point: 150-157°C Relative density (water = 1): 1.2 (25°C) Solubility in water, g/100mL: 0.03 (very poor) Vapour pressure, Pa at 25°C: negligible Flash point: 227°C c.c. Auto-ignition temperature: 510-570°C Octanol/water partition coefficient as log Pow: 3.32	The substance is toxic to aquatic organisms. It is strongly advised not to let the chemical enter into the environment.

NOTES

The substance is absorbed through the skin but no toxic effects were reported (2011)

ADDITIONAL INFORMATION

IPCS
International Programme on Chemical Safety

Prepared in the context of cooperation between the International Programme on Chemical Safety and the European Commission
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Source:

WHO International Programme on Chemical Safety and the European Commission 2004-2012. Available: http://www.ilo.org/dyn/icsc/showcard.display?p_lang=en&p_card_id=0634

PNT DBO (Biological oxygen demand)

1. OBJETIVO

Determinación de DBO (demanda bioquímica de oxígeno) mediante el equipo Oxi-direct con inóculo comercial.

2. RESPONSABILIDAD

El profesor/técnico de laboratorio es el encargado de la distribución y difusión de este PNT a todo el personal implicado.
La responsabilidad de aplicación de este procedimiento recae sobre la persona que realice la determinación, asi como de los registros que de este PNT se generan.

3. ALCANCE

Todo usuario de los laboratorios que realice la determinación DBO,así como el profesor responsable, en caso de que el usuario sea un alumno.

4. PPNNTT RELACIONADOS

- QI A900.01 : DBO Oxi-Direct

5. FINALIDAD DE USO

- Definición de la elaboración y procedimiento de uso de las disoluciones que intervienen en la determinación
- Concreción de los pasos a seguir para la determinación de DBO con inóculo comercial.

Garantizando el cumplimiento de las normativas y asegurando la coherencia entre PPNNTT.

6. PRINCIPIOS DEL MÉTODO. DEFINICIONES

Principios del método
La demanda Bioquímica de Oxígeno (DBO) es una prueba que determina los requerimientos relativos de oxígeno de aguas residuales, efluentes y aguas contaminadas, para su degradación biológica. Expresa el grado de contaminación de un agua residual por materia orgánica degradable por oxidación biológica en 5 días.
El instrumento determina el oxigeno consumido mediante la reducción de presión dentro del sistema de medición cerrado de DBO, empleando sensores de presión (determinación respirométrica de la DBO)
Durante la determinación DBO, las bacterias del agua introducida consumen el oxigeno disuelto en el agua. Este oxígeno consumido, es reemplazado por el oxigeno, que se encuentra en la cámara de aire de la botella de prueba. El dióxido de carbono producido simultáneamente por las mismas bacterias queda combinado químicamente con una

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solución de hidróxido de potasio, que se encuentra dentro de un pequeño depósito que hay en el cuello de la botella.
Por ello se produce un descenso de la presión, que es medida por los sensores. Este descenso de presión es directamente proporcional al consumo de oxigeno.

7. MATERIAL Y REACTIVOS

Los materiales y reactivos necesarios para realizar esta determinación son los siguientes:

Material	Caracteristicas	Cantidad
Frigotermostato	*	1
DBO Oxi-direct	*	1
pH-metro	*	1
Embudo aleman	*	1
Matraces aforados		varios

Reactivo	Caracteristicas	Cantidad
Tampón fosfato		Depende del número de muestras a analizar
Hidróxido de potassio	6 N	
Cloruro de calcio		
Sulfato de Magnesio		
Cloruro Férrico		
Hidróxido de sodio	1 N	
Àcido sulfúrico	1 N	
Àcid Glutàmic	300 mg/L	
Glucosa		
Inóculo	Comercial o fangos activados	

Antes de manipular los reactivos el usuario debe revisar las fichas de seguridad de las mismas y seguir las medidas que se especifican en las mismas para la correcta manipulación de reactivos

8. PROCEDIMIENTO

8.1. Preparación de reactivos (Tabla 1)

Nº	Nombre	Fórmula	Cantidad (g)	Volumen (mL)	pH
a)	Tampón fosfato	$NaH_2PO_4 \times H_2O$	23,81	100	6,8-7,2
		NH_4Cl	3,82		
b)	Hidróxido de potasio 6 N	KOH	39,6	100	N/A
c)	Cloruro de calcio	$CaCl_2$	2,77	100	N/A
d)	Sulfato de Magnesio	$MgSO_4 \times 7H_2O$	10,1	100	N/A

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e)	Cloruro Férrico	FeCl 3 x $6H_2O$	0,484	100	N/A
f)	Hidróxido de sodio 1 N	NaOH	4	100	N/A
g)	Ácido sulfúrico 1 N	H_2SO_4	7	250	N/A
h)	Patrón Àcid Glutàmico /Glucosa 300 mg/l	$C_5H_9NO_4$ i $C_6H_{12}O_6$	0,15 i 0,15	1000	6,8-7,2
i)	Agua de dilución	a), c), d), e)	1+1+1+1mL	1000	N/A
j)	inóculo (*)				

(*) Polyseed ®: depositar el contenido (polvo) de una cápsula en un matraz de 500ml y disolver con agua de dilución y). Agitar y airear durante 1 hora. Decantar el sobrenedant en un vaso de precipitados de 500ml limpio con una barra de agitación

NOTA IMPORTANTE: Para obtener mejores resultados, la disolución se tiene que utilizar antes de las seis horas posteriores a la rehidratación de la cápsula.

8.2. Preparación en función del rango de lectura (Tabla 2)

1) mg/L de DBO teórico	**2)** Volumen muestra, blanco o patrón	**3)** mL de cada solución (a,c,d,e)	**4)** Ajustar pH entre 6,5-7,5. pH real:	**5)** Volumen por botella DBO
0-40	498,0	0,5		426
0-80	498,0	0,5		358
0-200	249,0	0,25		242
0-400	199,2	0,2		155
7) mL inóculo	**8)** mL en la botella	**9)** gotas ATH	**11)** gotas de KOH	**12)** sensores
2	428	10	3-4	Posició:
2	360	10	3-4	Posició:
2	244	5	3-4	Posició:
2	157	5	3-4	Posició:

8.3. Descripción del procedimiento a seguir

1) mg/L teórico de DBO: Definir el valor de DBO que se espera obtener en la lectura de la muestra. Orientativo: entre el 68 i 80% de la DQO teórica.
2) Volumen muestra, blanco o patrón: mL a medir en función del rango de concentración a medir escogido (en el apartado 1)
3) mL de cada solución (a,c,d,e): Volumen que se tiene que añadir de cadauna de las disoluciones (del apartado 2)
4) Ajustar pH a 6,5-7,5: Ajustar con las disoluciones **g** o **f** en el interval indicado i anotar el valor real obtenido (tabla 2)

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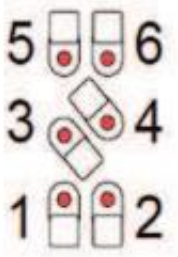
5) Volumen por botella DBO: Volumen de "**4)**" que se tiene que echar a cada botella de DBO
6) Atemperar: poner las muestras en el frigotermostato i esperar aproximadamente una hora
7) mL inóculo: Volumen de inóculo (disolución j) que se tiene que añadiren cada botella de DBO (Ver tabla 2)
8) mL muestra en la botella de DBO: Volumen total de muestra, patrón o blanco que habráen cada botella de DBO (suma de E+F de la tabla 2)
9) gotas ATH: Cantidad de gotas de inhibidor de nitrificación comercial que se tiene que añadir a cadauna de las botellas de DBO (ver tabla 2)
10) Introducir imán: poner a cada botella de DBO un iman limpio, evitando salpicaduras
11) Introducir gotas de KOH: Añadir 3-4 gotes de KOH en el interior de la cesta negra una vez ya se ha colocado sobre la botella de DBO
12) Enroscar los sensores: Colocar y enroscar los sensores de la DBO sobre cada botella i colocar cada una en una posición dentro del equipo. Anotar el orden (Ver Tabla 3)
13) Programar el equipo: Ver los siguientes apartados: Programación equipo, Lectura de muestras, Apagar el equipo.



	Descripción
Muestra 1	
Muestra 2	
Muestra 3	
Muestra 4	
Muestra 5	
Muestra 6	

Tabla 3. Descripción de las muestras y orden de colocación

8.4. Programación del equipo

1. Enchufar el equipo
2. Activar el lugar de medición de la botella con la muestra a analizar pulsando el botón del cabezal (Ver tabla 3).
3. Pulsar Start.
4. Seleccionar con los botones + y - el volumen de la muestra y el rango de medida.
5. Aceptar pulsando el botón Entero/Intro.
6. Mientras el equipo intenta activar la medida, indica "0.0.0" "y "0.0.0.0" (señal intermitente)
7. Si se ha activado correctamente, indica "000" "y "done"

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8.5. Lectura de muestras

Conectar, pulsando On/Off

Clicar el botón del cabezal que se quiere leer y pulsar READ para ver el valor actual de medida.

Si se quieren mirar todas al finalizar los 5 días, se tiene que ir pasando con el +.

Anotar los valores a la tabla 4

($mg\ O_2/L$)	Dia 1	Dia 2	Dia 3	Dia 4	Dia 5	Dia 6	Comentarios
Muestra 1							
Muestra 2							
Muestra 3							
Muestra 4							
Muestra 5							
Muestra 6							

Tabla 4. Registro del valor de la DBO obtenido

8.6. Apagar el equipo

Sacar las pilas si no se tiene que emplear el equipo durante un periodo largo de tiempo.

9. REGISTROS

Ver plantillas adjuntas:

- CRD.D.C.005.01 (control lectura PNT)
- CCH.D.C.006.01(control cambios PNT)

10. CONTROL DE COPIAS Y REGISTRO DE LECTURA

- Documento CRD.D.C.005.01 de control lectura para el presente PNT: PN/L/001/01
- Documento CCH.D.C.006.01 de control cambios para el presente PNT: PN/L/001/01

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ANEXO I

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UNIVERSITAT POLITÈCNICA DE CATALUNYA

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Centre de Enginyeria de Processos i Medi ambient (CEPIMA)
Laboratori de Processos

Hoja: de: | CODE CCH.D.C.006.01

REGISTRO DE CONTROL DE CAMBIOS

PNT		
Versión nº	Cambios realizados	Fecha

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ANEXO II

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CONTROL DE COPIAS Y REGISTRO DE LECTURA DEL PROCEDIMIENTO

Centre de Enginyeria de Processos i Medi ambient (CEPIMA)
Laboratori de Processos

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REGISTRO DE LECTURA DEL PROCEDIMIENTO

Nombre y Cargo	Codigo del PNT	Firma	Fecha

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Polyseed® Application Procedure, BOD_5 Seed Inoculum

InterLab®

POLYSEED®

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PolySeed® Application Procedure
BOD_5 Seed Inoculum

PolySeed® is a blend of broad spectrum bacteria designed specifically as a seed inoculum for the Biochemical Oxygen Demand (BOD_5) test as conducted in accordance to ***Standard Methods of the Examination of Water and Wastewater***. **PolySeed®** is an EPA approved BOD_5 seed inoculum that has been used to seed both municipal and industrial wastes for almost 35 years.

Overview: The following are the most important parts of the BOD_5 test. **First,** the BOD water must be made properly and stored at 20° C. **Next,** the PolySeed® solution must be properly rehydrated and tested to determine its' effect on the test (i.e., the Seed Control Factor – SCF). **Finally**, the seed inoculum must be tested against a known Glucose-Glutamic Acid (GGA) standard. With these tests in order, a very reliable and accepted BOD_5 test can be performed.

1st Step: Control – Dilution Water ("BOD Water"): Prepare the dilution water (some call it "BOD Water" or "Blank Water") in accordance with *Standard Methods*. Be sure to use fresh deionized water and remineralize with the appropriate nutrients and chemicals. Store the Control-Dilution water at 20° C until ready to use. Run a control ***"Blank"*** on the neat Control-Dilution water at 20° C along with the actual BOD test. To insure an acceptable final test the ***"Blank"*** must have an oxygen depletion of less than 0.2 mg/liter over the 5-day period. If you have any questions, refer to *Standard Methods,* InterLab's e-Guide Videos or our Frequently Asked Questions ("FAQ") page available at www.polyseed.com.

2nd Step: Seed Solution (i.e. "PolySeed® Solution"): To make the seed solution, place the entire contents of one PolySeed® capsule (discard the gelatin capsule) into 500ml of "DILUTION WATER" prepared in accordance to *Standard Methods* **(do not use DI water by itself)**. Normal dilutions are one PolySeed® capsule to 500ml of BOD water; however, the concentration of seed can be adjusted to compensate for variations in BOD water and established internal laboratory testing protocol. This seeded dilution water will be referred to as the "PolySeed® solution". ***Note: Bran, which acts as the carrier for the microorganisms, will neither dissolve nor inhibit microbial activity, but must be settled out of the PolySeed® solution prior to use.***

Next, aerate and stir the PolySeed® solution for 1 hour, then let the solution settle for 5 to 15 minutes. Finally, decant the supernatant carefully so as not to allow any bran in the biological solution. Pour the decanted PolySeed® solution in a clean 500ml beaker with a sterile stir bar, place on magnetic stirrer and gently stir for the remainder of the test. (Note: Our lab uses a Nalgene separatory funnel for this purpose) For best results, the PolySeed® solution should be used within 6 hours of rehydration of the capsule.

09/2012

Polyseed® Application Procedure, BOD_5 Seed Inoculum

3rd Step: Seed Control Factor ("SCF"): After following Step 2, carefully draw an aliquot from the PolySeed® solution. It is best to prepare the seed control using 15, 20, 25 & 30ml of PolySeed® solution; however, these aliquots may vary depending upon laboratory procedures. The resulting DO uptake should fall between 0.60 and 1.0 (see calculations below).

At the end of the 5-day test period calculate the **SEED CONTROL FACTOR ("SCF")** of the PolySeed® solution per *Standard Methods* by using [(D1 - D2) x f] where:

D1 = DO of seed control before incubation, mg/L
D2 = DO of seed control after incubation, mg/L and,
f = (Volume of seed in diluted sample)/(volume of seed in seed control)

Note: This can be automatically calculated using InterLab's BOD calculator.

4th Step: Glucose-Glutamic Acid Standard: After the glucose-glutamic acid (GGA) standard solution is prepared (refer to *Standard Methods* or our FAQ page at www.polyseed.com), use 4ml of PolySeed® solution for each BOD_5 bottle. Again, make sure there is no undissolved bran in the pipette. No other seed is required. (Note: PolySeed® solution volume can be adjusted to compensate for variations in DI water, laboratory procedures and established internal laboratory testing protocol.)

5th Step: BOD Sample Analysis: Prepare the live BOD samples in accordance with *Standard Methods*. Insure that the PolySeed® Solution is prepared and stirred in accordance with Step 2 above. Add 4mls of PolySeed® solution (this volume can be adjusted for varying BOD water) to each BOD_5 bottle when preparing the wastewater samples. No other seed is required. Follow *Standard Methods* procedures for incubation, seed correction, GGA, and dilution water preparation. When reporting results using PolySeed® it is best to use the BOD calculator located at www.polyseed.com or hand calculations in accordance with *Standard Methods*.

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09/2012

PNT TOC (Determination of total organic carbon)

PROCEDIMENTO NORMALIZADO DE TRABAJO
Grupo: Métodos de Trabajo (MT)

COT: DETERMINACIÓN DE CARBONO ORGÁNICO TOTAL

- Objetivo
- Responsabilidades
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- PPNNTT relacionados
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- Principios del método. Definiciones
- Material y reactivos
- Procedimiento
  - Descripción de los pasos a seguir para usar el analizador de carbono orgánico
  - Cálculo teórico de COT para verificar y calibrar
- Registros
- Control de copias y lectura
- Anexos
  - Control de cambios
  - Control lectura

COPIA CONTROLADA: NX

Realizada por:	Revisada por:	Aprobada por:
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Fecha: 02/04/12	Fecha: 09/04/12	Fecha: 17/04/12

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PNT TOC (Determination of total organic carbon)

1. OBJETIVO

Descripción de la metodologia a seguir para el correcto uso del analizador de Carbono Orgánico Total (COT) Shimadzu.

2. RESPONSABILIDAD

El profesor/técnico de laboratorio es el encargado de la distribución y difusión de este PNT a todo el personal implicado.
La responsabilidad de aplicación de este procedimiento recae sobre la persona que utilice el equipo, así como de los registros que de este PNT se generan.

3. ALCANCE

Todo usuario de los laboratorios que utilice en analizador de carbono orgánico total situado en el laboratorio de química analítica de la EUETIB, así como el profesor responsable, en caso de que el usuario sea un alumno.

4. PPNNTT RELACIONADOS

- PN/L/MT/002/01 : Proceso fotoFenton
- PN/L/MT/003/01 : Planta piloto
- QI A-754.02 : TOC. Propio de l'EUETIB

5. FINALIDAD DE USO

Definición de los pasos a seguir para la determinación del COT.

Garantizando el cumplimiento de las normativas y asegurando la coherencia entre PPNNTT.

6. PRINCIPIOS DEL MÉTODO. DEFINICIONES

Principios del método
El analizador de Carbono Orgánico Total (COT), basa su funcionamiento en una oxidación por combustión catalítica y detección por infrarrojo.
La señal del detector IR genera un pico, el área del cual es proporcional a la concentración de CT (carbono total) y IC (carbono inorgánico). Para realizar la medida de COT, se tiene que oxidar previamente la muestra con el fin de convertir todas las formas de carbono en CO_2. Para las mediciones de CI, el sistema añade automáticamente a las muestras ácido y las purga con aire de alta pureza. De este modo al disminuir el pH y burbujear aire se provoca la generación de CO_2 a partir de carbonatos y bicarbonatos, el cual es arrastrado por los aires al detector.

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PNT TOC (Determination of total organic carbon)

El volumen de muestra se inyecta sobre un catalizador que está a 680º C. El vapor de agua generado, que interfiere absorbiendo al infrarrojo, se elimina mediante condensación, enfriando el gas. La alta especificidad de la técnica de detección se debe de al hecho que los espectros infrarrojos son característicos de cada sustancia, presentando las muestras gaseosas espectros con máximos agudos.

La medida del COT se realiza por diferencia entre CT y IC.

7. MATERIAL Y REACTIVOS

Los materiales y reactivos necesarios para realizar esta determinación son los siguientes:

Material	Características	Cantidad
Analizador de COT	*	1
Vaso Precipitados	100-250mL	1
Recipiente para las muestras	*	Según uso

Reactivo	Características	Cantidad
Agua	MilliQ	Variable
HCl	37% o 2M	Variable
Aire sintético	Puro	En función del tiempo de uso

Antes de manipular los reactivos el usuario debe revisar las fichas de seguridad de las mismas y seguir las medidas que se especifican en las mismas para la correcta manipulación de reactivos

8. PROCEDIMIENTO

8.1. Descripción de los pasos a seguir para usar el analizador de carbono orgánico

1. **Rellenar** la hoja de registro de uso del COT.
2. **Abrir** la llave de paso AZUL del **aire sintético** (nivel a 2.5bar)
3. **Abrir** la tapa frontal del instrumento y hacer las siguientes **comprobaciones:**
    1. Nivel del depósito interno de agua: tiene que estar entre Lo i Hi, si no añadir agua MilliQ (Ver PNT TOC CODI: QI A-754.02)
    2. Nivel de agua del "drain pot" interno lleno hasta el máximo
4. **Cerrar** la puerta y hacer las siguientes **comprobaciones**:
    3. Nivel de agua de dilución del depósito externo
    4. Nivel de ácido clorhídrico 2M del depósito externo
5. **Encender** el equip con el botón **ON/OFF**. El equipo tarda 15-20 minutos en estabilizarse
6. **Análisis** de CT / CI / COT / NPOC
    5. Clicar "Measure Sample" del teclado.
    6. Para seleccionar los parámetros a medir: apretar F2 para los del CT, F3 para los del CI, F2 y F3 para los del COT y F5 para los del NPOC

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PNT TOC (Determination of total organic carbon)

7. Introducir los ***parámetros:***
   a. Introducir el **número de curva** (de 0 a 9) y apretar **ENTER.**
   b. **Nº Inyecciones (Inj #):** Apretar ▼ y introducir el nº de inyecciones que se quieren hacer y luego clicar **ENTER**. (**mínimo 2**)
   c. **Máximo de inyecciones (Max # oj Inj):** Introduicr el número máximo de inyecciones que se quieren hacer i presionar **ENTER.(entre 3 y 5**)
   d. Una vez seleccionadas las inyecciones clicar **NEXT**

NOTA: Si se quiere hacer una lectura continua en el mismo método de trabajo (**Same Mode Contin Meas**). Seleccionar ON cliclando ► . Se puede escoger entre On o OFF.

   e. Sumergir el **capilar en la muestra** cuando aparezca el mensaje: "Set Sample and press START button". **(Garantizar que durante toda la lectura el capilar queda sumergido en la muestra para evitar el deterioro del equipo)**
   f. **Verificar** que la lluz "Ready" está encendida y que la tecla "Start" parpadea.
   g. Apretar **Start.**
   h. Una vez acabada la lectura, clicar **F6** (Exit Mesurement) para salir.

7. Al acabar las inyecciones de las muestras, hacer **20 inyecciones de agua** ultrapura para limpiar el catalizador. Se mide con el CT que tarda aproximadamente **40 minutos**.
8. **Apagar** el equipo **SIEMPRE SIGUIENDO ESTE ORDEN**:
   a. Apretar F1 (Standby Options)
   b. Apretar F5 (Power off)
   c. Apretar F6 (execute) i
   d. F6 (yes). Sale un mensaje de parada: *It is a termination* i pasados unos 25 minutos el equipo se apaga
9. **Cerrar** la llave de paso del **aire** sintético
10. Volver a tapar el equipo para evitar que le entre polvo. Si no se ha apagado el ventilador del equipo (lateral izquierdo), tapar el equipo sin cubrir esa parte (para evitar que se estropee)

8.2. Cálculo teórico de COT para verificar y calibrar

Se puede calcular el valor teórico de carbono orgánico total que tendria que tener una sustancia conociendo:

- El número de carbonos que tiene
- Su peso molecular

Mediante la aplicación de la siguiente ecuación:

Cálculo teórico de COT= [sustancia, en ppm] x 12 x número de C de la sustancia x (1/peso molecular de la sustancia).

El cálculo del COT teórico sirve para escoger las rectas con las que se harán las medidas (puesto que el equipo dispone de rectas hechas a diferentes rangos de concentración) y para comprobar que los valores experimentales obtenidos concuerdan con los teóricos y, por lo tanto, verificar que se están obteniendo resultados coherentes.

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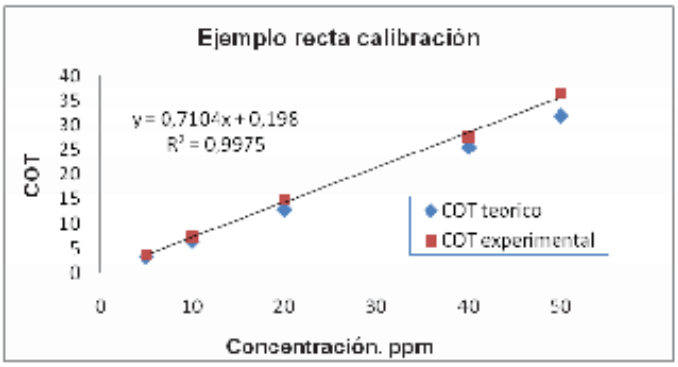
PNT TOC (Determination of total organic carbon)

8.2.1. Ejemplo: recta de calibración de paracetamol

El paracetamol , tiene la siguiente fórmula molecular $C_8H_9NO_2$, con 8 átomos de carbono y 151g/mol, por lo tanto, se puede calcular y comparar el valor teórico de COT con los valores medidos experimentalmente tal como se observa en la siguiguiente tabla.

CONCENTRACIÓN (ppm)	TOC TEÒRICO(*)	TOC EXPERIMENTAL	Diferencia COT (ppm)
50	31,79	36,46	4,67
40	25,43	27,50	2,07
20	12,72	14,75	2,04
10	6,36	7,34	0,98
5	3,18	3,73	0,55

(*) El cálculo teórico de COT para el patrón de 50ppm se obtiene de la siguiente manera:= (50 ppm) x (12) x (8 átomos de C de la sustancia) x (1mol /151g).= 31.79



9. REGISTROS

Ver plantillas adjuntas:

- CRD.D.C.005.01 (control lectura PNT)
- CCH.D.C.006.01(control cambios PNT)

10. CONTROL DE COPIAS Y REGISTRO DE LECTURA

- Documento CRD.D.C.005.01 de control lectura para el presente PNT: PN/L/001/01
- Documento CCH.D.C.006.01 de control cambios para el presente PNT: PN/L/001/01

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PNT H_2O_2 (Determination of hydrogen peroxide)

1. OBJETIVO

Determinación de H_2O_2 mediante espectrofotometria Vis. Se puede aplicar para seguir la evolución de las reacciones de degradación mediante procesos de oxidación avanzada (en los que interviene el peróxido de hidrógeno).

2. RESPONSABILIDAD

El profesor/técnico de laboratorio es el encargado de la distribución y difusión de este PNT a todo el personal implicado.
La responsabilidad de aplicación de este procedimiento recae sobre la persona que realice la determinación, asi como de los registros que de este PNT se generan.

3. ALCANCE

Todo usuario de los laboratorios que realice la determinación de peróxido de hidrógeno,así como el profesor responsable, en caso de que el usuario sea un alumno.

4. PPNNTT RELACIONADOS

- PN/L/MT/002/01 : Proceso fotoFenton
- PN/L/MT/003/01 : Planta piloto
- PN/L/MT/008/01 : Espectrofotometria UV-Vis

5. FINALIDAD DE USO

- Definición de la elaboración y procedimiento de uso de las disoluciones que intervienen en la determinación
- Concreción de los pasos a seguir para la determinación del peróxido de hidrogeno.

Garantizando el cumplimiento de las normativas y asegurando la coherencia entre PPNNTT.

6. PRINCIPIOS DEL MÉTODO. DEFINICIONES

Principios del método
Determinación de peróxido de hidrógeno durante reacciones de foto degradación: La concentración de peróxido residual durante la degradación de compuestos orgánicos en técnicas de oxidación avanzadas es un parámetro importante a evaluar.
El método está basado en la reacción de peróxido de hidrógeno con metavanadato amónico en medio ácido, que provoca la formación del catión peroxovanadio (VO_2^{3+}), que es de un color rojo-naranja y presenta un máximo de absorbancia en 450 nm.

$$VO_3^- + 4H^+ + H_2O_2 \rightarrow VO_2^{3+} + 3H_2O \; VO_3^- + 4H^+ + H_2O_2 \rightarrow VO_2^{3+} + 3H_2O$$

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PNT H_2O_2 (Determination of hydrogen peroxide)

En la reacción, el vanadato (VO^{3-}) se oxida a peroxovanadio (VO_2^{3+}) y el peróxido de hidrógeno se reduce a agua.

En el método se añade un exceso de vanadato amónico para que reaccione todo el peróxido. Como resultado la concentración final de peroxovanadio es igual a la concentración inicial de peróxido de hidrógeno por la estequiometria de la reacción y cuantificando la concentración de peroxovanadio final se sabe cual era la concentración de peróxido de hidrógeno inicial. $[VO^{3-}] = [H_2O_2]$.
La absorción a 450 nm es proporcional a la $[VO^{3}]$ lo que permite su cuantificación mediante espectrofotometria UV-visible.
Nota: La valoración del H_2O_2 con permanganato es un método convencional estandarizado, sin embargo, no es recomendable usar este método para el seguimiento de las muestras de reacciones Foto-Fenton porque el Fe^{2+} reacciona también con el permanganato, lo cual interfiere en la determinación de peróxido.

7. MATERIAL Y REACTIVOS

Los materiales y reactivos necesarios para realizar esta determinación son los siguientes:

Material	Caracteristicas	Cantidad
Matraz aforado	100 ml	8
Vaso Precipitados	250 ml	3
Vaso Precipitados	25 ml	1
Pipeta aforada	10 ml	1
Pipeta aforada	1 ml	1
Pipeta graduada	50 ml	1
Pipeta graduada	2 ml	1
Pipeta graduada	5 ml	1
Pipeta Pasteur	3 ml	1
Pesa substancias	-	1
Espátula y pera o pipum	*	1
Manta calefactora	-	1
Balanza	-	1
Espectrofotómetro UV-Vis	Hitachi U-2001	1
Cubetas para el espectrofotómetro	Paso óptico 1cm	2

Reactivo	Caracteristicas	Cantidad
Vanadato	98,5% pureza	7,5 g
H_2SO_4	96% pureza	50 ml
H_2O_2	30% p/v	1 ml

Antes de manipular los reactivos el usuario debe revisar las fichas de seguridad de las mismas y seguir las medidas que se especifican en las mismas para la correcta manipulación de reactivos

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PNT H_2O_2 (Determination of hydrogen peroxide)

8. PROCEDIMIENTO

8.1. Preparación de reactivos

a) Disolución H_2SO_4 [9M]

-Para preparar 100 ml de esta disolución, se tienen que calcular los ml de H_2SO_4 .que se tienen que añadir. en función de su pureza. Ejemplo de cálculo para preparar 100mL de disolución partiendo de un reactivo con una pureza del 96% y una densidad de 1,84 $g \cdot ml^{-1}$:

$$100ml \times \frac{9moles}{1000ml} \times \frac{98,08g_{puro}}{1mol} \times \frac{100g_{reactivo}}{96g_{puro}} \times \frac{ml}{1,84g} = 49,97ml$$

Instrucciones

-En un vaso de precipitados poner unos 20 ml de agua desionizada

- A continuación añadir lentamente el volumen calculado del reactivo sulfúrico concentrado.

- Cuando se haya enfriado enrasar con agua en un matraz aforado del volumen correspondiente.

b) Disolución H_2SO_4 [0,58M] y de Vanadato Amónico [0,062 M]. Cálculos:

$$\frac{0,062moles}{1L} \times \frac{116,98g_{puro}}{1mol} \times \frac{100g_{reactivo}}{98,5g_{puro}} = 7,3632g\ NH_4VO_3$$

$$\frac{0,58moles}{1L} \times \frac{98,08g_{puro}}{1mol} \times \frac{100g_{reactivo}}{96g_{puro}} \times \frac{ml}{1,84g} = 32,20ml\ H_2SO_4$$

*Como la estequiometria de la reacción es 2 moles de H_2SO_4 por 1 mol de NH_4VO_3 se tiene entonces que poner 64,40 ml de H_2SO_4

Instrucciones

- Pesar los 7,3632gramos de vanadato amónico

- Añadir los 7,3632g de vanadato amónico sobre los 64,4mL de ácido sulfúrico 9 M agitando continuamente con un agitador magnético porqué sinó el sólido se engancha en el fondo y no se puede disolver..

- Calentar a 50 ºC con agitación hasta disolución del vanadato amónico.

- Cuando se haya enfriado enrasar con agua en un matraz aforado de 1L.

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PNT H_2O_2 (Determination of hydrogen peroxide)

8.2. Preparación de disoluciones patrón para recta de calibración

Se denominará:

X→ Disolución H_2SO_4 [0,58M] y Vanadato Amonico [0,062 M] previamente preparada

A→ 1ml de H_2O_2 (30% p/v) enrasado con agua a 100ml

a) Preparación

Para preparar los patrones se seguirá la siguiente tabla:

Patrón	ml X	ml A	ppm H_2O_2
Blanco	10	0	0
1	10	1	30
2	10	2	60
3	10	3	90
4	10	4	120
5	10	5	150

Todos los patrones se enrasan con aguadesionizada hasta llevarlos a 100 ml en un matraz aforado.

8.3. Preparación de las muestras para la lectura en espectrofotómetro

Se explica un ejemplo del procedimiento a seguir con matraces de **10 mL** aunque se puede hacer con otros volúmenes. Se tiene que mirar SIEMPRE que la **concentración de metavanadato añadido sea mayor que la de peróxido de hidrógeno que puede contener la muestra**. Los cálculos se muestran a continuación:

- Concentración de VO_3^- =0.0062M x (volumen añadido en el matraz / volumen matraz)
- Concentración de H_2O_2 = $[H_2O_2]$(mg/L) x (1g/1000mg) x (1 mol/34g) x (volumen añadido en el matraz / volumen del matráz)

Ejemplo:

- Coger el número de matraces de 10 mL necesarios para medir el peróxido de hidrógeno en el tiempo de experimento realizado (por ejemplo 8)
- Añadir 1.1mL de la disolución de metavanadato amónico 0.0062M (ver 8.1). Ahora se tiene una concentración de 0.0062M x 1.1mL / 10mL = 6.82 x 10^{-4} M, por lo que como MÁXIMO, podré añadir esta concentración de H_2O_2.

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PNT H_2O_2 (Determination of hydrogen peroxide)

- Suponiendo que a la disolución muestra problema le hayamos añadido 400mg/L de H_2O_2, deberemos añadir 0.5mL para asegurarnos que se para la reacción en el caso que no se haya descompuesto el H_2O_2.

  NOTA: a medida que avanza la reacción, se descompone el H_2O_2 i el volumen a coger puede ser mayor.

8.4. Lectura de la absorbancia en el espectrofotómetro

Pasos a seguir para hacer la lectura en el espectrofotómetro Perkin Elmer:

-Encender la impresora y después el espectrofotómetro, y esperar 5minutos

-Pulsar la tecla "method". Seleccionar el método 4

-Pulsar la tecla "parameter" hasta que aparezcala longitud de onda. Verificar que se encuentra a 450 nm.En caso contrario, entrar este valor con el teclado y clicar enter

- Seguir pulsando la tecla "parameter" hasta que aparezca backcorr. Verificar que se encuentra en "yes" o entrar este valor con las flechas laterales del teclado.

- Clicar la tecla "stop"

-Abrir la tapa del espectro, poner una cubeta con solución blanco en cada portamuestras.

-Pulsar la tecla "start"

-Cuando en la pantalla se vea 0.000 abs, se quita la primera cubeta y se va llenando con los patrones, es decir primer patrón 1, anotar abs que aparece en la pantalla del espectrofotómetro, patrón 2, anotar abs, y asi sucesivamente.

*Importante antes de poner la cubeta con el patrón, limpiar la cubeta con el mismo patrón, es decir se llena, y se tira, un par de veces, y se limpian con papel suave (tissues) ambas caras de la cubeta para impedir que el polvo o las huellas interfieran en la lectura. Se tiene que introducir la cubeta en el protamuestras del espectrofotómetro siempre en la misma dirección.para evitar variaciones en la lectura

-Cuando se hayan anotado todas las absorbancias se cierran la impresora y el espectrofotómetro.

Si se utiliza el espectrofotómetro de UV-Vis Agilent consultar PN/L/MT/008/01

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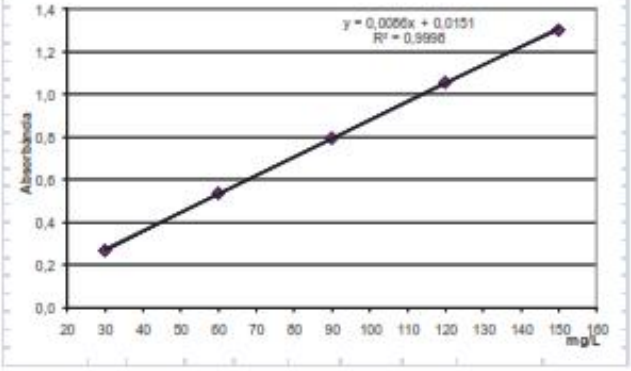
PNT H_2O_2 (Determination of hydrogen peroxide)

8.5. Ejemplo de curva de calibrado

ppm	Absorbancies				Desv. st.	%Desv.
	Exp. 1	Exp. 2	Exp. 3	Mitjana		
30	0,273	0,262		0,268	0,0076	2,91
60	0,548	0,524	0,537	0,536	0,0120	2,24
90	0,812	0,763	0,769	0,792	0,0153	1,93
120	1,075	1,084	1,055	1,059	0,0225	2,13
150	1,315	1,279	1,309	1,301	0,0193	1,48



8.6. Verificación del estado de las disoluciones y del peróxido de hidrogeno

Las disoluciones para esta determinación se conservan en nevera y pueden ser utilizadas durante amplios periodos. Antes de utilzarlas se tiene que verificar que no han sido alteradas. Por otro lado el peroxido de hidrogeno debe ser verificado antes de utilizarlo para realizar un experimento en el que su valor exacto sea importante. En ambos casos una comprobación ràpida puede ser:

1. Preparar los patrones de 60 y 120 ppm.
2. Verificar que las absorbancias leídas coinciden con las de la curva de calibración

8.7. Normalización del peróxido de hidrógeno

Cada vez que se empiece a usar una botella nueva de peróxido de hidrógeno, se tiene que hacer la normalización de este producto para determinar su %.

Procedimiento:

1. **Preparación de la solución de $KMnO_4$**

- Pesar de 3,2 a 3,5 g de $KMnO_4$
- Pasarlo a un vaso de precipitados de 1500 mL
- Agregar 1 L de agua destilada
- Tapar el vaso con un vidrio de reloj

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PNT H_2O_2 (Determination of hydrogen peroxide)

- Hervir la solución suavemente por 15-30 min
- Dejar enfriar a temperatura ambiente
- Filtrar la solución fría con un filtro de Gooch de porosidad fina
- Guardar en un frasco ámbar o mantener en la oscuridad

2. Estandarización de la solución de $KMnO_4$ con $Na_2C_2O_4$ (0,02 N)

- Pesar con exactitud 0,1 g de $Na_2C_2O_4$ seco en un erlenmeyer de 250 mL
- Disolver en 75 mL de ácido sulfúrico 1,5 N
- Calentar la solución recién preparada entre 80 y 90ºC y titular lentamente con la solución de $KMnO_4$ agitando constantemente con movimientos circulares
- El punto final lo determina la aparición de un color rosa tenue que persiste por lo menos 30 segundos
- La temperatura no debe descender por debajo de 60ºC
- Realice tres determinaciones y calcula su promedio aritmético ($\overline{V_K}$)

3. Valoración del H_2O_2

- Disuelva 1 mL de peróxido de hidrógeno en 100 mL de agua destilada
- Coloque 1 mL de esta disolución anterior en un erlenmeyer de 250 mL
- Diluya con 25 mL de agua destilada y mezcle bien
- Añada 20 mL de ácido sulfúrico 2 N
- Titule en frio con la solución valorada de $KMnO_4$ hasta la aparición del primer tono rosado permanente
- Realice tres determinaciones y calcule su promedio aritmético ($\overline{V_{K2}}$)

Pesos equivalentes:

- H_2SO_4: 49,04
- $KMnO_4$: 31,61
- $Na_2C_2O_4$: 67
- H_2O_2: 17

Cálculos

- Normalidad del $KMnO_4$

$$\overline{V_K}N_K = V_C N_C \Rightarrow N_K = \frac{0,075L(0,02eq/L)}{\overline{V_K}}$$

- Concentración del peróxido de hidrógeno

$$\overline{V_{K2}}N_K = eqH_2O_2 \Rightarrow \%H_2O_2 = \frac{eqH_2O_2(17g/eq)}{0,1mL}100$$

4. Anotar en la botella el resultado obtenido y la fecha del día de la normalización

ESTE DOCUMENTO NO SE PUEDE REPRODUCIR SIN LA DEBIDA AUTORIZACIÓN

PNT H_2O_2 (Determination of hydrogen peroxide)

9. REGISTROS

Ver plantillas adjuntas:

- CRD.D.C.005.01 (control lectura PNT)
- CCH.D.C.006.01(control cambios PNT)

10. CONTROL DE COPIAS Y REGISTRO DE LECTURA

- Documento CRD.D.C.005.01 de control lectura para el presente PNT: PN/L/001/01
- Documento CCH.D.C.006.01 de control cambios para el presente PNT: PN/L/001/01

ESTE DOCUMENTO NO SE PUEDE REPRODUCIR SIN LA DEBIDA AUTORIZACIÓN

Preliminary experiments - BOD_5 and BOD_7 /BOD_8 results of BPA solutions
(2.5; 5.0; 7.5; 10.0; 20.0; 30.0 mg/L)

BOD analyses were implemented in accordance to the instructions of UPC laboratory using four pieces of equipment placed in two frigothermostat. BOD_5 was analyzed from solutions of BPA having different concentrations and two different sample sizes were used (Chapter 3.3). Results data is presented in this appendix 6 on page 2. Unexpectedly there was a problem with two devices (2 and 4) that did not start measuring properly. Because this was realized in the next morning, new solutions were made and new measurement started with two devices one day later (12 samples).

Samples of 360 mL (devices 1 and 3) were fine and measurement worked properly. With the new sample solutions (428 mL), the second measurement also worked properly, but the temperature of the frigothermostat was higher than +20ºC and it was not stable. Starting one day later, it also meant that when reading BOD_7 value, it was actually BOD_8 of devices 1 and 3. Because the Lovibond Oxidirect system was set to measure 5 days value, this was recorded automatically. The value of following days had to be read manually. For practical reasons the final value had to be read on the same day, so for comparison of the results it has to be noticed that devices 1 and 3 have BOD_8 value and devices 2 and 4 have BOD_7 value. Anyway, for the main purpose of the study the BOD_5 value was used.

Figures 1 and 2 show the results of the BOD process. Oxygen demand was highest during the first days and moderated after that. All measurements had quite similar pattern and there were no clear inconsistency, so it can be concluded that the test worked the way it should have been. If there had been problems, such as air leakage, it could have been seen in the results.

Preliminary experiments - BOD_5 and BOD_7 /BOD_8 results of BPA solutions (2.5; 5.0; 7.5; 10.0; 20.0; 30.0 mg/L)

Equipment 1

Bottle	d1	d2	d3	d4	d5	d6	d8	pH (real)	BPA Conc	Sample size	capsule	OBS:
1.1	10	20	35	37	39		43	7,02	0,0	360	4	(0,5ml too much food)
1.2	7	16	27	28	29		39	7,04	2,5	360	4	
1.3	4	9	25	27	28		37	7,05	5,0	360	4	
1.4	10	29	35	38	39		42	7,02	seed control	360	15	
1.5	3	3	4	3	3		3	6,95	7,5	360	4	(no food, mistake!)
1.6	2	11	22	23	24		25	6,79	10,0	360	4	

Equipment 2.

Bottle	d1	d2	d3	d4	d5	d6	d7	pH (real)	BPA Conc	Sample size	
2.1	8	29	32	40	44		45	6,72	0,0	428	4
2.2	14	30	32	34	35		37	6,8	2,5	428	4
2.3	16	32	33	35	35		38	6,83	5,0	428	4
2.4	12	30	33	36	40		43	6,72	s. control	428	15
2.5	10	26	27	28	29		31	7,3	7,5	428	4
2.6	10	26	26	27	27		29	7,12	10,0	428	4

Equipment 3.

Bottle	d1	d2	d3	d4	d5	d6	d8	pH (real)	BPA Conc	Sample size	
3.1	14	33	36	39	41		43	7,02	0,0	360	4
3.2	5	17	26	27	28		28	6,98	20,0	360	4
3.3	7	26	29	31	32		32	7	30,0	360	4
3.4	13	33	38	41	43		50	7,02	s. control	360	25
3.5	12	31	35	37	39		40	7,02	s. control	360	20
3.6	-										

Equipment 4.

Bottle	d1	d2	d3	d4	d5	d6	d7	pH (real)	BPA Conc	Sample size		
4.1	12	30	33	40	45		47	6,72	0,0	428	4	
4.2	12	28	29	31	34		35	6,86	20,0	428	4	
4.3	6	24	24	26	29		29	7,4	30,0	428	4	
4.4	12	33	36	39	43		43	6,72	s. control	428	20	
4.5	6	5	5	6	9		6	6,72	0,0	428	4	0,0 no food
4.6	7	43	48	OFL	OFL		OFL	6,86	20,0	428	4	20,0 double food

Biodegradability (BOD5) and BPA concentrations, aliment 50 ppm

	0 (no aliment)	0*	2,5	5	7,5	10	20	30	7,5 (no food)
Equipments 1+3 (360 ml)		40	29	28	-	24	28	32	3
Equipments 2+4 (428 ml)	9	44	35	35	29	27	34	29	
corrected 1+3 (-blank=7)		33	22	21		17	21	25	
corrected 2+4 (-blank=9)	0	35	26	26	20	18	25	20	
mean	**9**	**34**	**24**	**23,5**	**20**	**17,5**	**23**	**22,5**	

* averidge of two measurements

BOD of the blanc: 7

Aliment: 4,5 mL (for 360mL) and 5,4 mL (for 480 mL) was measured in the bottles. Aliment was prepared as 4 g/L solution, where 2 g of Glutamic acid and 2 g of Glucose were diluted into a one litre of deionised water.

Eq. 1 & 3 have actually BOD_8 value instead of BOD_7, because the day 7 value was not possible to read.

Graphs of the preliminary BOD tests - BOD_5 and BOD_7 results of BPA solutions (2.5; 5.0; 7.5; 10.0; 20.0; 30.0 mg/L)

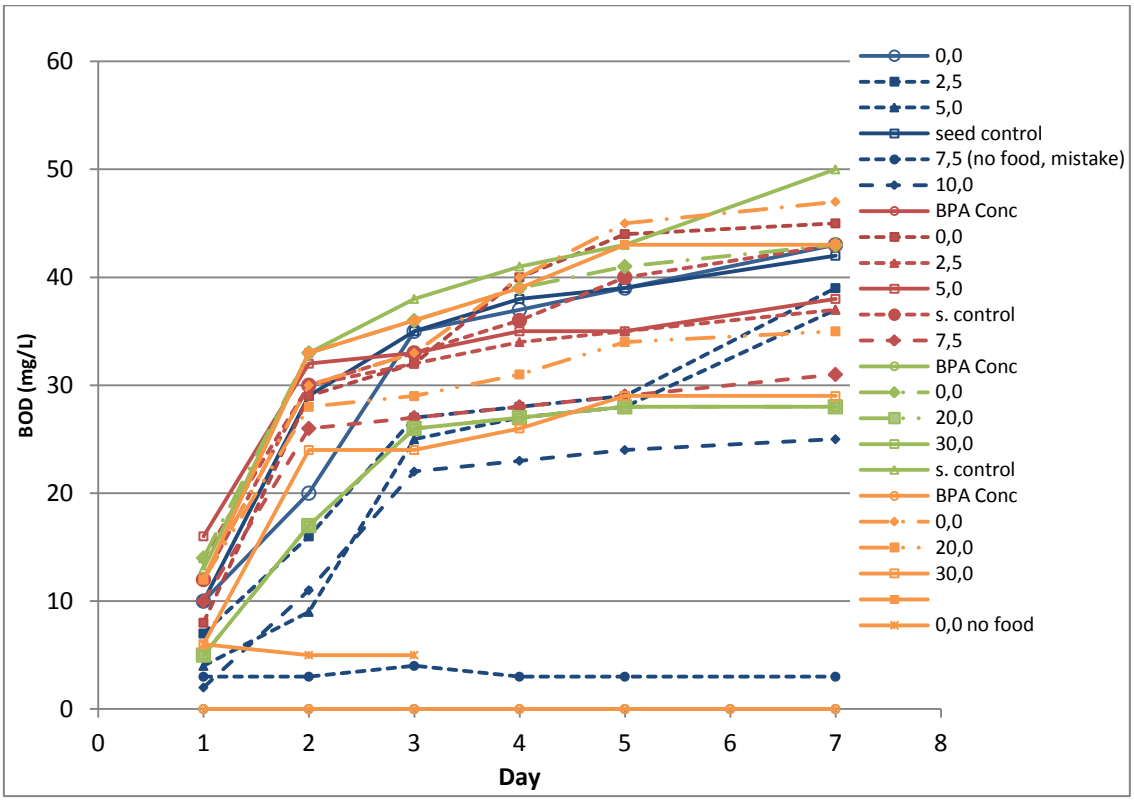


Figure 1. BOD_5 and BOD_7 of the preliminary samples. Different colours present different BOD devices; blue = equipment 1 (360 mL), red = eq.2 (428 mL), green = eq.3 (360 mL) and yellow eq.4 (428mL). It should be noted that devices 1 and 3 gave actually BOD_8 value, because it was not possible to read them on the day 7 (see the table, Appendix 6, 2/3).

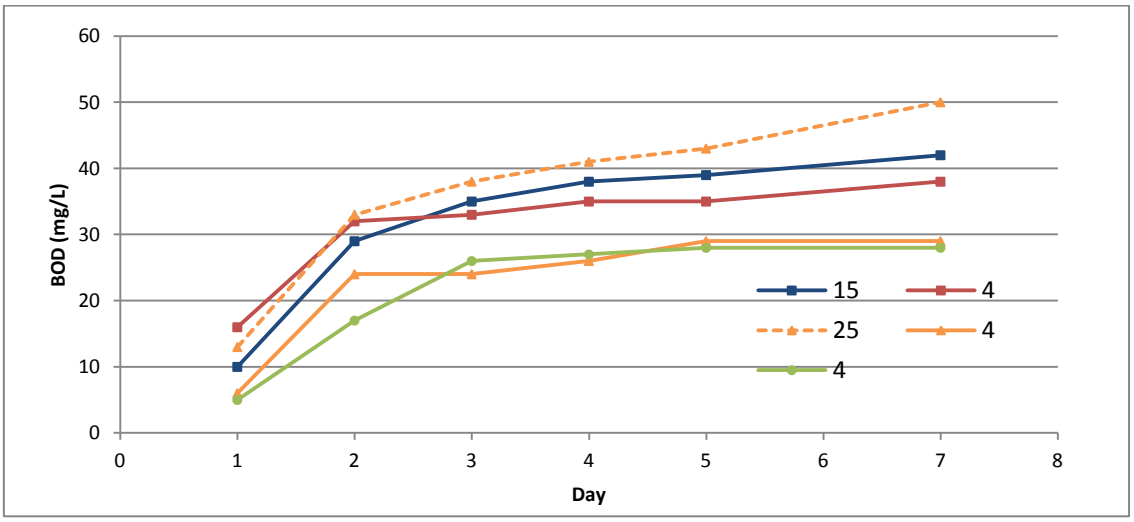


Figure 2. Seed control samples of 15, 20 and 25 mL of seed solution. Seed control factor (SCF) was calculated only from the experiments but not from these preliminary BOD tests (see SCF of the experiments, appendix 7).

Seed Control Factor calculations as part of BOD analysis, reliability

Seed controls were taken in accordance to manufacturer's instructions in order to find out that the Polyseed inoculum was working properly (InterLab® 2012). The effect of the seed is minor when analysing BOD_5 and is not taken into account in these calculations although it also could be subtracted from the final BOD. Three seed controls were measured using BOD-water (having same minerals and aliment quantity as samples), but adding 15, 20 and 25 mL of seed solution. Results of the preliminary BOD tests are not included, because of practical reasons, dissolved oxygen was not measured from those samples.

From samples of the experiments, dissolved oxygen was measured with handheld DO-meter (Crison 330i/340i) before and after BOD test from the blank and seed control samples. After value was not possible to take after 5 days, because also BOD_7 value was taken, so DO was measured after 7 days. It can be assumed that the value is slightly higher than it would have been after 5 days. 25 mL control was excluded, because there were no ATH drops left for the sample and the results were not consistent. Seed control factor (SCF) was calculated using following formula:

D1-D2 * f = 0.60 - 1.0	D1 = DO of seed control before incubation, mg/L D2 = DO of seed control after incubation, mg/L f = volume of seed in diluted sample/ volume of seed in seed control

f= ml of Polyseed used in samples/GGA 4,0

Table 1. Dissolved O_2 measurements for the blank and control samples.

Bottle	Sample	DO before BOD	DO after BOD7	DO before - DO after	
1.1.	blank	5,47	5,34	0,13	
1.4.	control 15 ml	5,49	4,18	1,31	
2.1.	blank	5,33	5,26	0,07	
2.4.	control 20 ml	5,38	3,12	2,26	
3.1.	blank	-	4,56	-	
3.4.	control 25 ml	7,83	2,51	5,32	EXCLUDED

Table 2. The results of the seed control factor calculations.

Seed Control:				
sample volume:	360 ml			
volume of seed	15	20	25	ml
BOD7 (Oxi-direct)	29	47	30	mg/l
D1 -D2	1,31	2,26	5,32	mg/l
calculated BOD	31,44	40,68	76,61	
Seed control factor*	**0,35**	**0,45**	**0,85**	
			EXCL.	

* must be between 0,6-1,0

Table 3. The measured DO of the blank samples. Depletion was less than the limit and can be considered reliable

Blanks:

Bottle	Sample	DO before - DO after
1.1.	blank	0,13
2.1.	blank	0,07
Mean value**		**0,10**

** must be < 0,2 mg/l

The 25 mL seed control sample had to be excluded, because it was made from different BOD-water than all other controls and also ATH drops finished, so this sample had none. Probably because of this, DO and BOD values were not consistent neither reliable. According to the results, seed control factor of 15 and 20 mL samples was too low (0.35-0.45), but on the other hand the calculated BOD of these samples (31 and 41 mg/L) was quite close to the measured values of Lovibond Oxidirect (29 and 47 mg/L).

Conclusion: DO of the blanks (0.1 mg/L) was below the limit (0.2 mg/L), which supports the reliability in terms of the BOD water and bottles. The actual mean BOD_5 value of the blanks (7 mg/L) was used to correct the BOD_5 results of the samples (Chapter 5.2.1). Effect of the seed was considered so minor (0.4) that it was not included BOD calculations. SCF was slightly too small, but overall this test was considered reliable enough for the purpose of the study.

Table 4. Polyseed calculator sheet offered by InterLab® gives the same information than calculations in Tables 1 and 3 (InterLab 2014b).

Product Lot # :		**Technician:**	TK				
Date In:	5.11.2014.	**Date Out:**	11.11.2014.				
Temp In:	20,00	**Temp Out:**	19,9				
Note: This calculator does not take out SCF's that fall outside of the 0.6-1.0 range. Nor does it take out GGA's that fall outside of the 198 ± 30.5 range					**Notes:**		
1. BLANKS: Checks the BOD water & BOD b							
DEP MUST BE <0.2 mgL							
Bottle #	Initial DO	Final DO	Depletion				
1.1.	5,47	5,34	0,13		Only blanks and seed controls were done, not enough place for all controls. GGA was freshly made and considered reliable.		
2.1.	5,33	5,26	0,07				
	AVERAGE BLANKS:		**0,10**	OK!			
2. SEEDED CONTROLS (SCF): Calculates the effect c							
f= ml of PS used in samples/GC	4,0				Must be between: 0.6-1.0		
Bottle #	(mL) of PolySeed	Initial DO B1	Final DO B2	n B1 - B2	% Dep.	BOD	SCF (B1-B2)f
1.4.	15	5,49	4,18	1,3100	23,9%	31,4	0,3493
2.4.	20	5,38	3,12	2,2600	42,0%	40,7	0,4520
3.4.	25	7,83	2,51	5,3200	67,9%	76,6	0,8512
	30			0,0000	#JAKO/0!	0,0	0,0000
				Dep. Must be > 2mg/L	**AVERAGE SCF:**		**0,4131**

Details and TOC of the BPA solutions [30 mg/L] used in experiments

Table 1. Details of the BPA solutions used in the photo-Fenton experiments. TOC of the BPA solutions (30 mg/L) for experiments, diluted from 120 mg/L of BPA solution. Solutions I-V were made in one solution, VI-IX another one (*) and the last solutions X-XI had the third solution (**).

Exp.	Name	Date	BPA Solution	TOC of BPA Solution		
				TC	IC	TOC
A1	*BPA_30_40.25_5.0_ON*	*20.10.*	*II excl.*	*39,28*	*15,93*	*23,35*
A2	BPA_30_40.25_5.0_ON_2	28.10.	V	25,84	1,177	24,66
B1	BPA_30_40.25_10.0_ON	14.10.	I	25,12	0,674	24,45
B2	BPA_30_40.25_10.0_ON_2	28.10.	V	25,84	1,177	24,66
B3	BPA_30_40.25_10.0_ON_3	30.10.	VII*	24,81	0,744	24,07
C1	*BPA_30_161_5.0_ON*	*20.10.*	*II excl.*	*39,28*	*15,93*	*23,35*
C2	BPA_30_161_5.0_ON_2	22.10.	IV	24,87	1,098	23,77
C3	BPA_30_161_5.0_ON_3	28.10.	VI*	25,1	0,826	24,27
D1	BPA_30_161_10.0_ON	13.10.	I	25,12	0,674	24,45
D2	BPA_30_161_10.0_ON_2	22.10.	IV	24,87	1,098	23,77
D3	BPA_30_161_10.0_ON_3	31.10.	VIII*	24,99	0,757	24,23
D4	BPA_30_161_10.0_ON_4	11.11.	IX*	23,99	0,646	23,34
E	BPA_30_100.63_7.5_ON	22.10.	III	25,76	0,751	25,01
F	BPA_30_100.63_7.5_ON_2	22.10.	III	25,76	0,751	25,01
G	BPA_30_100.63_7.5_ON_3	28.10.	VI*	25,1	0,826	24,27
E2	BPA_30_100.63_7.5_ON_4	30.10.	VII*	24,81	0,744	24,07
H	BPA_30_100.63_3.96_ON	31.10.	VIII*	24,99	0,757	24,23
I	BPA_30_100.63_11.04_ON	13.11.	X**	23,57	0,735	22,84
J	BPA_30_15.24_7.5_ON	18.11.	XI**	24,17	0,808	23,362
K	BPA_30_186.01_7.5_ON	18.11.	XI**	24,17	0,808	23,362

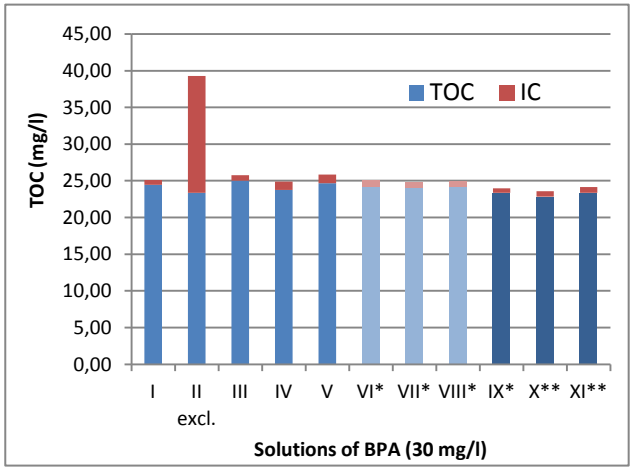


Figure 1. TOC of the BPA solutions (30 mg/L) for the experiments.

BPA solutions for experiments (30 mg/L, diluted from 120 mg/L):

Date	Solution no	TC	IC	TOC
13.10.	I	25,12	0,674	24,45
20.10.	*II excl.*	*39,28*	*15,93*	*23,35*
22.10.	III	25,76	0,751	25,01
22.10.	IV	24,87	1,098	23,77
28.10.	V	25,84	1,177	24,66
28.10.	VI*	25,1	0,826	24,27
30.10.	VII*	24,81	0,744	24,07
31.10.	VIII*	24,99	0,757	24,23
11.11.	IX*	23,99	0,646	23,34
13.11.	X**	23,57	0,735	22,84
18.11.	XI**	24,17	0,808	23,36

Adjusted pH during the experiment

Exp.	Name	initial pH	before 5 min	after 5 min	after 10 min	after 20 min	after 30 min	after 45 min	after 60 min	final pH	Duration	code	
A1	*BPA_30_40.25_5.0_ON*	*2,85*								*2,87*	*60*	*NA*	excl. Solution, TOC!
A2	BPA_30_40.25_5.0_ON_2	2,86							3,01 to 2.9 4	2.9 3	90	A	
B1	BPA_30_40.25_10.0_ON	2.9 0	2,77 (NA)		2,76 (NA)	2,79(NA)	2,80 (NA)			2,80	60	NA	
B2	BPA_30_40.25_10.0_ON_2	2,85			2,76 to 2,86					2.9 4	90	A	
B3	BPA_30_40.25_10.0_ON_3	2.9 2			2,79 to 2,85					2,86	90	A	
C1	BPA_30_161_5.0_ON	2,83				2,74 to 2,80				2.9 2	60	A	
C2	BPA_30_161_5.0_ON_2	2.9 4								2.9 8	90	NA	
C3	BPA_30_161_5.0_ON_3	2,88	2,75 to 2,89						3,02 to 2.9 5	2.9 4	90	A	
D1	BPA_30_161_10.0_ON	2.9 7								-	90	NM	excl. Solution, TOC!
D2	BPA_30_161_10.0_ON_2	2.9 0			2,69 to 2,82					2.9 9	90	A	
D3	BPA_30_161_10.0_ON_3	2,88	2,77 to 2,86				3,03 to 2.9 0			2,88	90	A	
D4	BPA_30_161_10.0_ON_4	2,89	2.59 to2,87		2,78 - 2,84			3,2 to 2.9 8	3,09 to 2,87	2.9 3	90	A	
E	BPA_30_100.63_7.5_ON	2,88			2,77 to 2,82					2,89	60	A	
F	BPA_30_100.63_7.5_ON_2	2,89			2,74 to 2,83					2.9 6	90	A	
G	BPA_30_100.63_7.5_ON_3	2,85		2,76 to 2,82						2.9 8	90	A	
E2	BPA_30_100.63_7.5_ON_4	2.9 4		2,75 to 2.9 4		3,02 to 2,89		3,03 to 2.9 4		2.9 4	90	A	
H	BPA_30_100.63_3.96_ON	2.9 2			2,76 to 2,87					2.9 6	90	A	
I	BPA_30_100.63_11.04_ON	2.9 0		2,69 to 2,89		3,02 to 2,86		3,01 to 2,87		2.9 6	90	A	
J	BPA_30_15.24_7.5_ON	2,84								2,80	60	NA	
K	BPA_30_186.01_7.5_ON	2.9 2	2,73 to 2,86					3,03 to 2,83		2.9 3	90	A	

* NA = not adjusted, A = adjusted, NM = not monitored nor adjusted

Monitored / adjusted temperature

Exp.	Name	initial t	5	*10*	20	30	45	60	75	90	+-
A1	*BPA_30_40.25_5.0_ON*	**25,0**	-	-	-	-	-	-			
A2	BPA_30_40.25_5.0_ON_2	**25,0**	-	26,0	-	-	25,0	25,0	25,0	25,2	1,0
B1	BPA_30_40.25_10.0_ON	**25,0**	-	-	-	-	-	-			
B2	BPA_30_40.25_10.0_ON_2	**25,0**	-	27,0	27,0	26,0	26,0	26,0			2.0
B3	BPA_30_40.25_10.0_ON_3	**25,5**	27,0	27,0	27,0	27,0	27,0	27,0	27,1	27,1	1,6
C1	BPA_30_161_5.0_ON	**25,0**	-	-	-	-	-	-			
C2	BPA_30_161_5.0_ON_2	**26,2**	26,5	-	26,9	27,0	27,2	27,2	27,2	28,0	1,9
C3	BPA_30_161_5.0_ON_3	**25,5**	25,5	26,0	25,0	25,0	25,0	25,0	26,5		1,0
D1	BPA_30_161_10.0_ON	**25,0**	-	-	-	-	-	-			
D2	BPA_30_161_10.0_ON_2	**24,9**			25,4	25,5	25,8	26,2	26,3	26,7	1,8
D3	BPA_30_161_10.0_ON_3	**26,0**	26,0 to 25,0		26,1	26,8	27,0	26,9	26,9	27,0	2.0
D4	BPA_30_161_10.0_ON_4	**25,0**		26,0			26,0	26,0	26,1	26,1	1,1
E	BPA_30_100.63_7.5_ON	**26,9**	26,9	27,0	27,0	-	-	-			0,1
F	BPA_30_100.63_7.5_ON_2	**26,5**	26,5	26,5	-	-	-	27,0	27,2	27,3	1,3
G	BPA_30_100.63_7.5_ON_3	**26,0**	26,0	25,0	24,5	25,0	25,0	25,0	25,2	26,0	1.5
E2	BPA_30_100.63_7.5_ON_4	**26,2**	26,5	27,0	27,0	27.5 to 26,9		26,9	26,9	27,0	27,0	1,3
H	BPA_30_100.63_3.96_ON	**25,5**	26,5	27,0	27,0	27,0	27,1	27,1	27,8	27,9	2.4
I	BPA_30_100.63_11.04_ON	**25,5**	25,5	25,5	25,2	25,5	25,3	25,6	25,9	25,9	0,7
J	BPA_30_15.24_7.5_ON	**25,0**	25,0	25,0	25,0	25,0	25,0	25,0	25,0	25,0	0
K	BPA_30_186.01_7.5_ON	**25,0**	25,5	25,5	25,5	25,2	25,2	25,1	25,0	25,0	0,5

* NM = not monitored nor adjusted, NNA= no need to adjust, NA= not adjusted, A= adjusted(cooled)

The results of BOD experiments, data

Equipment 1

Bottle	Sample	d1	d2	d3	d4	d5	d6	d7	pH (real)	caps ule	real ATH	
1.1	blank	6	7	7	7	7		7	6,66	4	no	
1.2	A2	7	24	32	35	37		38	6,89	4	5	
1.3	B2	8	13	27	30	31		31	6,75	4	5	
1.4	control 15	3	12	26	27	28		29	6,66	15	5	
1.5	C3	6	22	29	30	30		31	7,28	4	5	
1.6	E	5	20	27	30	31		31	6,83	4	5	

Equipment 2.

Bottle	Sample	d1	d2	d3	d4	d5	d6	d7	pH (real)	caps ule	real ATH	
2.1	blank	7	7	7	7	7		7	6,66	4	no	
2.2	B3	10	19	38	42	44		46	7,17	4	5	
2.3	D3	1	8	23	24	24		25	6,88	4	5	
2.4	control 20	16	36	41	44	45		47	6,66	20	5	
2.5	F	6	12	30	32	32		32	7,27	4	5	
2.6	BPA_30	17	18	28	29	29		29	6,7	4	5	half the food

Equipment 3.

Bottle	Sample	d1	d2	d3	d4	d5	d6	d7	pH (real)	caps ule	real ATH	
3.1	blank	4	5	5	5	5		5	6,66	4	no	
3.2	D2	5	28	31	31	33		35	6,93	4	5	
3.3	G	7	14	30	31	31		33	7,37	4	5	
3.4	control 25											
3.5	E2				EXCLUDED							
3.6	BPA_30_2											half the food

Obs. ATH-drops (to prevent nitrification) were finished , so only 5 drops instead of 10 were added into each bottle. The last three samples were without drops, results lower and therefore excluded.

Equipment 1

Bottle	Sample	d5	d5-blank
1.1	blank	7	0
1.2	A2	37	30
1.3	B2	31	24
1.4	control 15	28	21
1.5	C3	30	23
1.6	E	31	24

Equipment 2.

Bottle	Sample	d5	d5-blank
2.1	blank	7	0
2.2	B3	44	37
2.3	D3	24	17
2.4	control 20	45	38
2.5	F	32	25
2.6	BPA_30	29	22

Equipment 3.

Bottle	Sample	d5	d5-blank
3.1	blank	5	-2
3.2	D2	33	26
3.3	G	31	24
3.4	control 25		
3.5	E2	EXCLUDED	
3.6	BPA_30		

BOD of the blanks:	Eq 1	eq 2	Eq 3	Eq 4 (prel. Test)	mean
(used in correction)	**7**	**7**	**5**	**9**	**7**

Aliment: 4,5 mL of GGA solution (4 g/L) was measured into each bottle. Aliment was freshly prepared by diluting 2 g of Glutamic acid and 2 g of Glucose into 1 litre of deionised water.

The results of BOD experiments, graphs

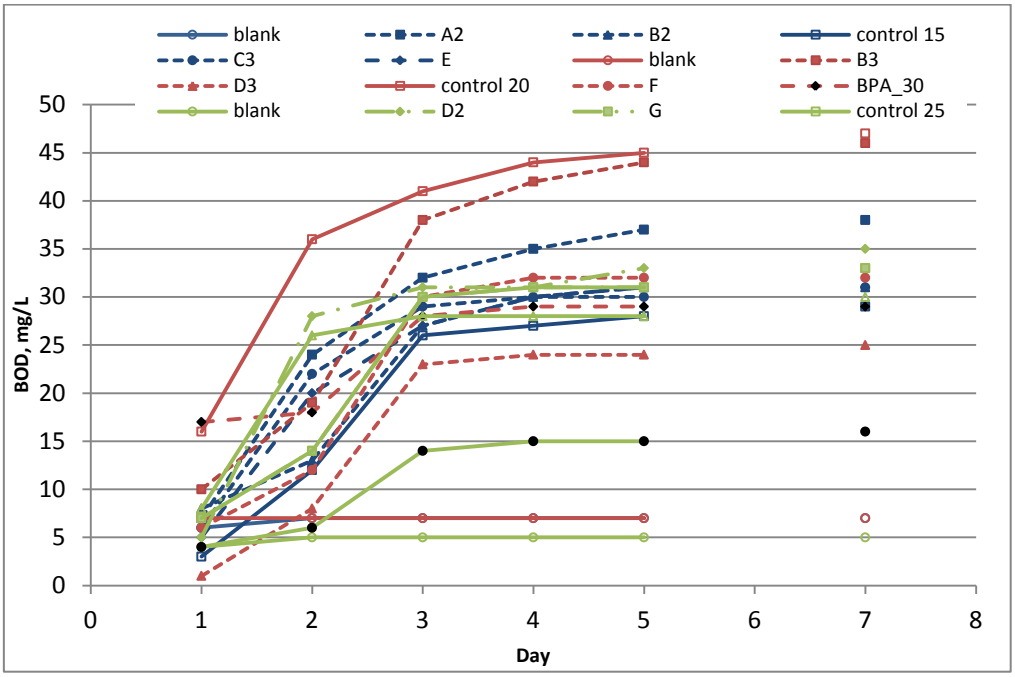
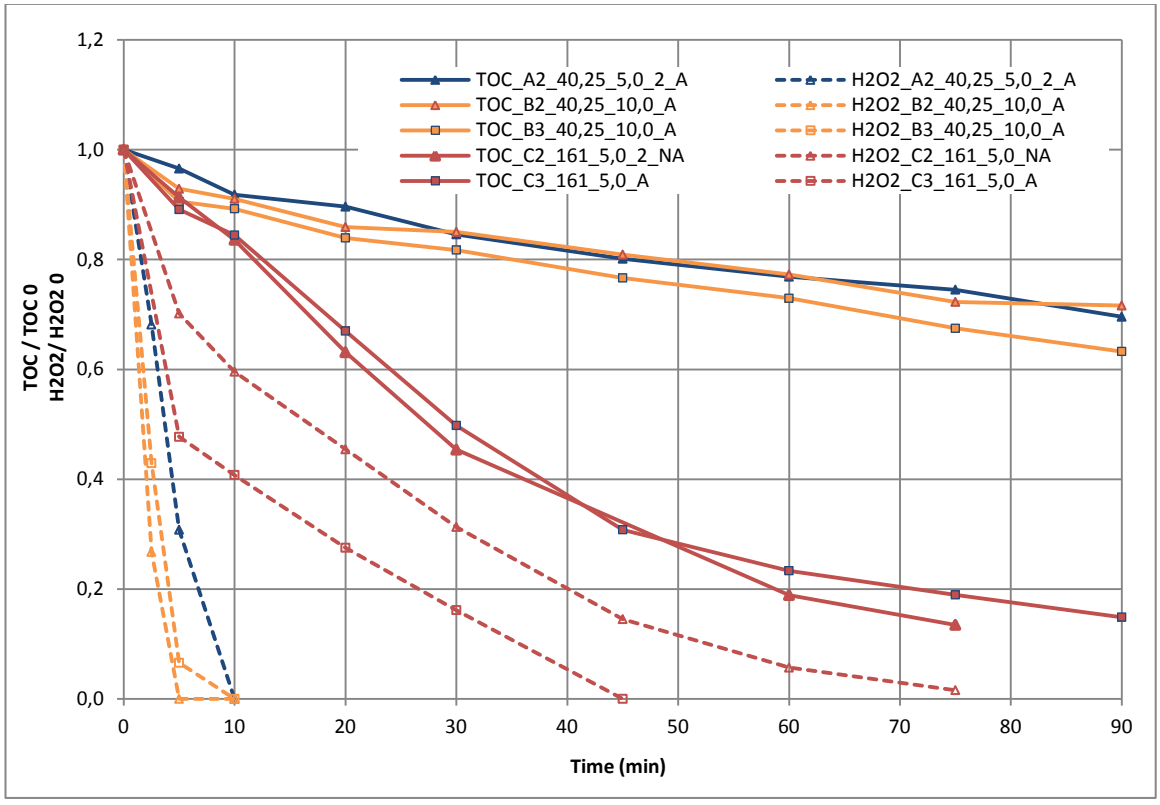


Figure 1. BOD5 and BOD7 values of each day. All curves apart from blanks are rising as they should, more significantly during first days. There is not a big difference in 5 and 7 day values, although the 5-day value is the one used in this study.

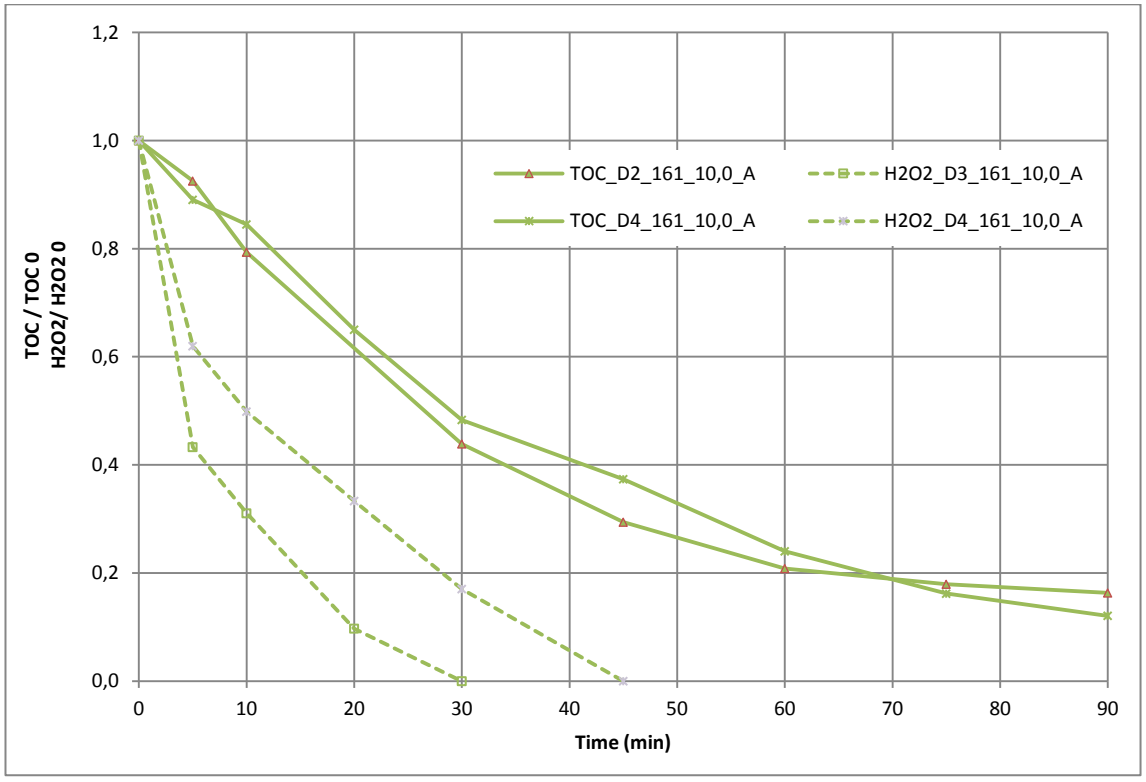
TOC results, experiments
A_40.25_5.0
B_40.25_10.0
C_161_5.0

t (min)	A2_TOC /TOC0	A2_H202 /H2O20	B2_TOC /TOC0	B2_H2O2 /H2O20	B3_TOC /TOC0	B3_H2O2 /H2O20	C2_TOC /TOC0	C2_H202 /H2O20	C3_TOC /TOC0	C3_H202 /H2O20
0	1,000	1,0000	1,0000	1,0000	1,0000	1,0000	1,0000	1,0000	1,0000	1,0000
2,5		0,6815		0,2676		0,4291				
5	0,966	0,3079	0,9288	0,0000	0,9055	0,0656	0,9133	0,7017	0,8910	0,4771
10	0,918	0,0000	0,9106	0,0000	0,8925	0,0000	0,8359	0,5957	0,8445	0,4077
15										
20	0,896		0,8591		0,8393		0,6311	0,4544	0,6698	0,2751
25										
30	0,846		0,8503		0,8173		0,4538	0,3130	0,4978	0,1615
35										
40										
45	0,801		0,8091		0,7664			0,1451	0,3079	0,0000
50										
55										
60	0,769		0,7727		0,7295		0,1889	0,0568	0,2333	
65										
70										
75	0,745		0,7228		0,6747		0,1346	0,0158	0,1893	
80										
85										
90	0,696		0,7162		0,6326				0,1488	



TOC results, experiments
D_161_10.0

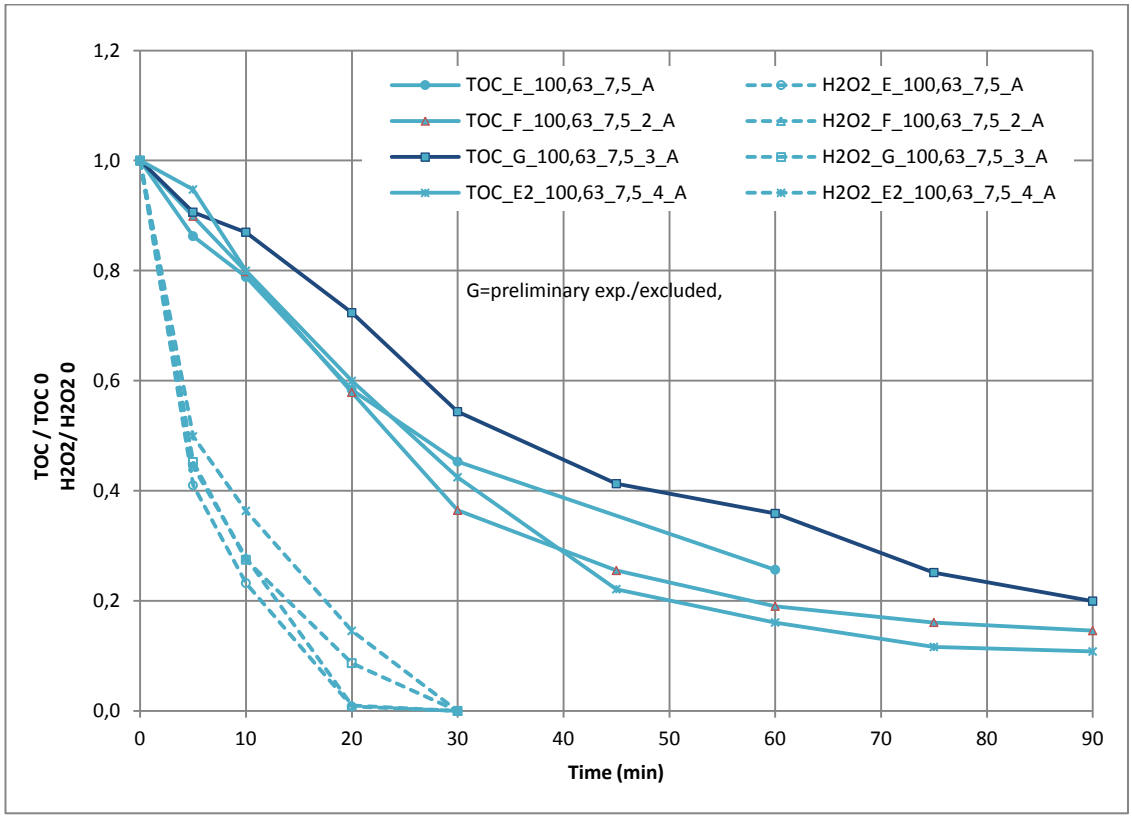
t (min)	D2_TOC /TOC0	D2_H2O2 /H2O20	D4_TOC /TOC0	D4_H2O2 /H2O20
0	1,0000	1,0000	1,0000	1,0000
2,5				
5	0,9251	0,2878	0,8903	0,6197
10	0,7934	0,1805	0,8448	0,4985
15				
20		0,0379	0,6500	0,3332
25				
30	0,4388	0,0000	0,4832	0,1704
35				
40				
45	0,2941		0,3735	
50				
55				
60	0,2082		0,2401	
65				
70				
75	0,1792		0,1618	
80				
85				
90	0,1631		0,1208	



TOC results, experiments E-G_100.63_7.5

preliminary exp. /excluded

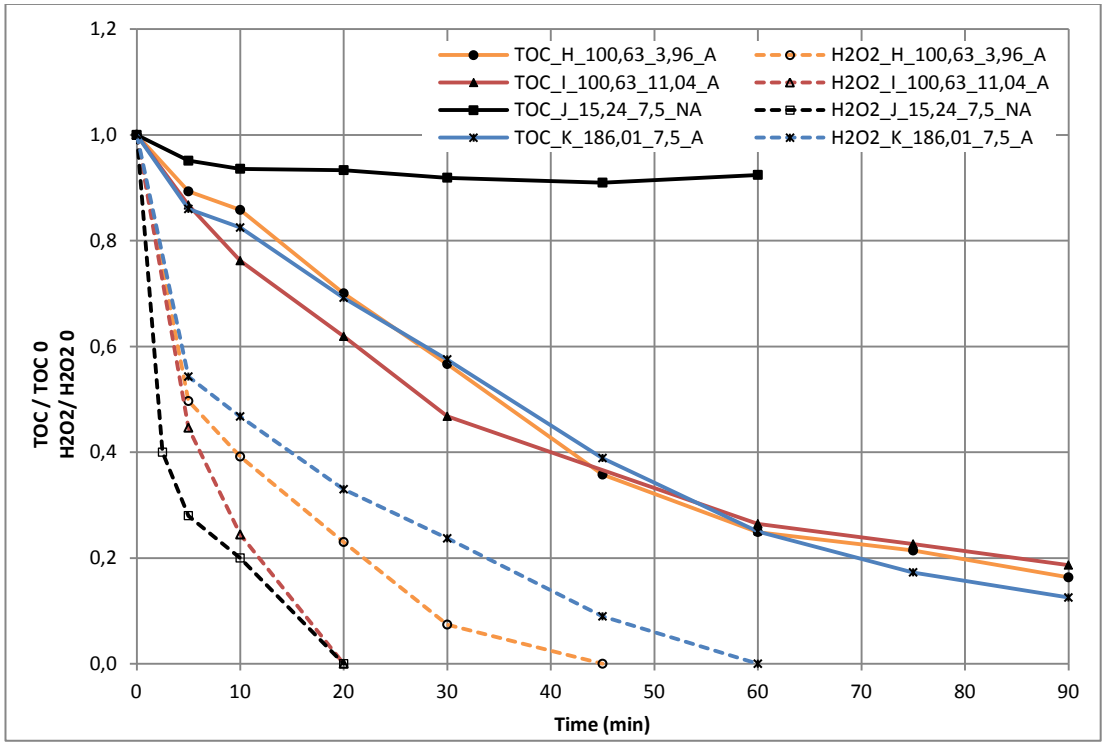
t (min)	E_TOC /TOC0	E_H2O2 /H2O20	E2_TOC /TOC0	E2_H2O2 /H2O20	F_TOC /TOC0	F_H2O2 /H2O20	G_TOC /TOC0	G_H2O2 /H2O20
0	1,0000	1,0000	1,0000	1,0000	1,0000	1,0000	1,0000	1,0000
2,5								
5	0,8627	0,4099	0,9473	0,4988	0,8988	0,4442	0,9061	0,4523
10	0,7884	0,2322	0,7996	0,3635	0,7983	0,2787	0,8697	0,2746
15								
20	0,5835	0,0081	0,5994	0,1454	0,5787	0,0101	0,7235	0,0868
25								
30	0,4530	0,0000	0,4245	0,0000	0,3645	0,0000	0,5437	0,0000
35								
40								
45			0,2211		0,2551		0,4128	
50								
55								
60	0,2565		0,1607		0,1902		0,3587	
65								
70								
75			0,1161		0,1608		0,2514	
80								
85								
90			0,1079		0,1458		0,1996	



TOC results, experiments

H_100.63_3.96
I_100.63_11.04
J_15.24_7.5
K_186.01_7.5

t (min)	H_TOC /TOC0	H_H2O2 /H2O20	I_TOC /TOC0	I_H2O2 /H2O20	J_TOC /TOC0	J_H2O2 /H2O20	K_TOC /TOC0	K_H2O2 /H2O20
0	1,0000	1,0000	1,0000	1,0000	1,0000	1,0000	1,0000	1,0000
2,5						0,4000		
5	0,8931	0,4967	0,8674	0,4463	0,9512	0,2800	0,8599	0,5429
10	0,8581	0,3917	0,7621	0,2443	0,9359	0,2000	0,8248	0,4675
15								
20	0,7004	0,2302	0,6190	0,0000	0,9333	0,0000	0,6921	0,3299
25								
30	0,5667	0,0740	0,4678		0,9191		0,5753	0,2370
35								
40								
45	0,3577	0,0000			0,9097		0,3889	0,0896
50								
55								
60	0,2491		0,2649		0,9244		0,2503	0,0000
65								
70								
75	0,2141		0,2265				0,1728	
80								
85								
90	0,1634		0,1866				0,1252	



Toxicity results, *Escherichia Coli*

Table 1. Growth maximum without contaminant (controls). Mean of all controls was used to normalize all the results.

Controls - Growth maximum without contaminant (measured value)						**mean of all controls**	
0,428	0,397	0,462	0,357	0,328	0,357	0,4195	
0,478	0,502	0,477	0,408	0,388	0,452		
Same values normalized by the mean value (value/mean*100)						**mean**	**SD**
102.03	94,64	110,13	85,10	78,19	85,10	100	13,31
113,95	119,67	113,71	97,26	92.49	107,75		

Table 2. Normalized results of two different sets of BPA solutions (value/mean of the controls*100).

C_{BPA} mg/mL	**1st BPA samples**			**Mean**	**SD**
0	107,27	115,85	94,64	105,92	10,67
2.5	79,14	80,33	84,86	81,45	3,02
5	95,83	95,35	100,60	97,26	2.9 0
7,5	77,47	76,04	84,15	79,22	4,33
10	100,83	92.01	106,08	99,64	7,11
20	91.5 4	88,44	106,56	95,51	9,69
30	99,88	89,15	102.9 8	97,34	7,26
C_{BPA} mg/mL	**2nd BPA samples**			**Mean**	**SD**
0	110,61	106,20	119,67	112,16	6,87
2.5	118,00	110,85	133,49	120,78	11.5 8
5	95,35	100,83	111,08	102.42	7,99
7,5	100,36	100,12	112,75	104,41	7,23
10	97,97	99,17	103,46	100,20	2,88
20	106,56	97,26	108,22	104,01	5,91
30	104,17	99,88	123,24	109,10	12.44

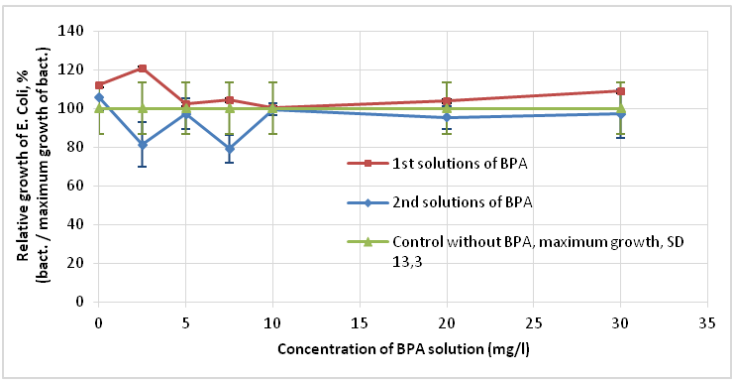


Figure 1. The toxicity results of BPA solutions using *E. Coli*.

Table 3. Normalized toxicity results of the experiments using *E. coli* (value/mean of the controls). Value in the table is the mean of all three measurements. Because 0-sample was not taken (the first one only after 5 minutes), the value used is BPA (30mg/L), which does not contain H_2O_2.

	K_186.01_7.5		**E2_100.63_7.5_4**		**D4_161_10.0**		**I_100.63_11.04**	
T(min)	mean	SD	mean	SD	mean	SD	mean	SD
0	1,032	0,112	1,032	0,112	1,032	0,112	1,032	0,112
5	0,802	0,153	0,919	0,086	0,616	0,248	0,999	0,065
10	0,643	0,073	0,953	0,028	0,648	0,159	0,901	0,039
20	0,841	0,170	0,871	0,015	0,777	0,158	0,837	0,097
30	1,147	0,414	1,005	0,256	0,806	0,167	1,032	0,169
45	1,004	0,094	0,953	0,133	0,916	0,046	0,858	0,025
60	0,985	0,090	0,899	0,060	1,053	0,202	0,930	0,113
75	1,215	0,342	0,929	0,064	1,224	0,533	0,875	0,005
90	1,005	0,064	0,913	0,037	1,009	0,103	0,979	0,028

	B1_40.25_10.0		**H_100.63_3.96**		**C3_161_5.0**		**A2_40.25_5.0**	
T(min)	mean	SD	mean	SD	mean	SD	mean	SD
0	1,032	0,112	1,032	0,112	1,032	0,112	1,032	0,112
5	1.5 26	0,643	0,761	0,205	0,886	0,340	1,340	0,164
10	1,074	0,137	0,793	0,044	1,002	0,069	1,074	0,130
20	1,292	0,180	0,980	0,072	0,973	0,089	1,109	0,079
30	1,139	0,141	1,010	0,047	0,981	0,114	0,970	0,086
45	1,135	0,107	1,000	0,058	1,035	0,091	1,086	0,161
60	1,023	0,060	1,014	0,019	0,997	0,049	1,054	0,038
75			1,025	0,033	0,892	0,313	0,957	0,081
90			1,222	0,112	0,746	0,200	1,035	0,066

	B2_40.25_10.0		**D3_161_10.0**		**C2_161_5.0**		**F_100.63_7.5_2**	
T (min)	mean	SD	mean	SD	mean	SD	mean	SD
0	1,032	0,112	1,032	0,112	1,032	0,112	1,032	0,112
5	1,282	0,086	1,195	0,074	0,578	0,117	1,356	0,282
10	1,243	0,312	1,026	0,057	0,577	0,206	1,076	0,113
20	1,081	0,172	0,875	0,033	0,450	0,067	1,130	0,244
30	1,062	0,064	1,356	0,544	0,688	0,031	0,964	0,053
45	1,166	0,070	1,302	0,483	0,994	0,166	1,309	0,180
60	1,063	0,160	1,303	0,404	0,909	0,001	1,103	0,294
75	1,230	0,121	1,110	0,102	0,988	0,122	1,112	0,185
90	1,405	0,124	1,056	0,129	1,078	0,156	1,165	0,305

	D2_161_10.0		**B3_40.25_10.0**		**E_100.63_7.5**		**J_15.24_7.5**	
T(min)	mean	SD	mean	SD	mean	SD	mean	SD
0	1,032	0,112	1,032	0,112	1,032	0,112	1,032	0,112
5	1,084	0,062	1,055	0,020	1,198	0,095	1,165	0,256
10	1,031	0,072	1,050	0,065	1,244	0,270	0,819	0,097
20	1,097	0,215	0,992	0,056	1,174	0,221	1,078	0,172
30	1,132	0,145	0,996	0,033	1,076	0,124	0,892	0,124
45	1,102	0,123	0,962	0,019	1,228	0,391	0,992	0,105
60	1,121	0,136	0,971	0,004	1,109	0,053	0,961	0,128
75	1,207	0,083	1,012	0,070			0,828	0,040
90	1,249	0,364	1,056	0,004			0,992	0,078

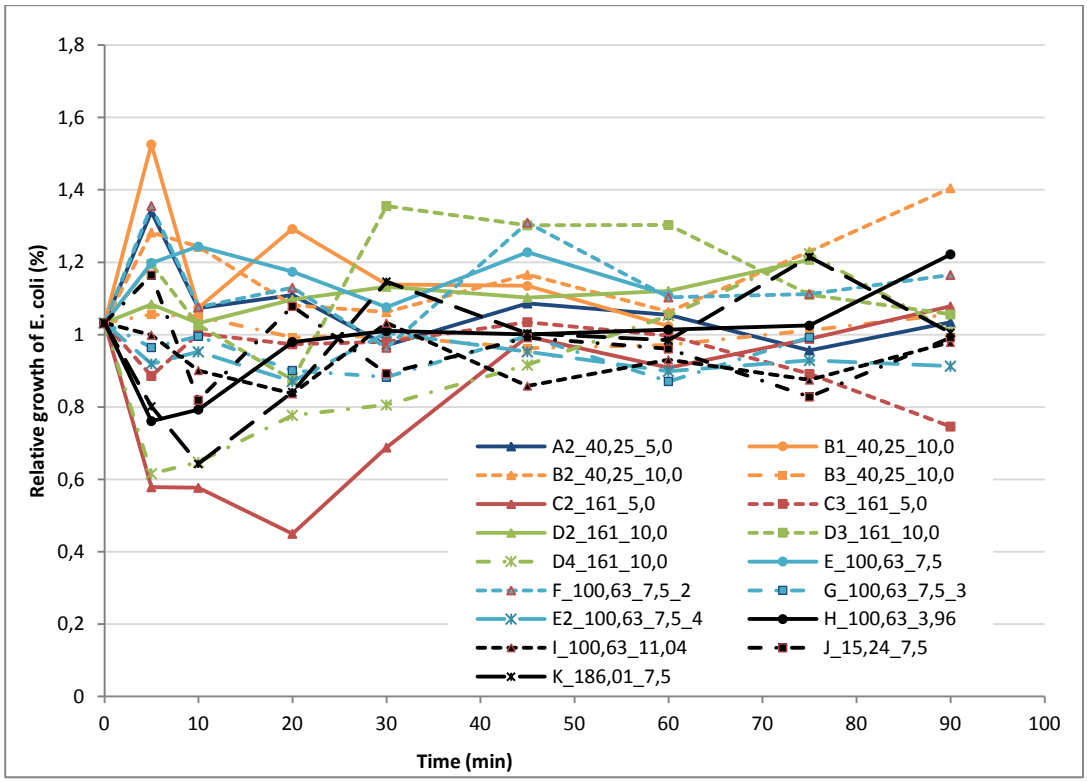


Figure 2. The relative growth of the *E. coli*. This means bacteria growth divided by the maximum growth of the bacteria (= the mean of controls). It should be noted that 0-value is the value of BPA [30 mg/L] without H_2O_2, because samples were not taken in the beginning of the experiment (the first sample was taken after 5 minutes).

Toxicity results, *Staphylococcus epidermidis*

Table 1. Growth maximum without contaminant (controls). Mean of all controls was used to normalize all the results.

Controls - Growth maximum without contaminant (measured value)						**mean of all controls**	
0,589	0,588	0,537	0,576	0,596	0,551	0,6697	
0,754	0,706	0,844	0,782	0,765	0,748		
Same values normalized by the mean value (value/mean*100)						**mean**	**SD**
87,95	87,80	80,19	86,01	89,00	82,28	100,00	15,96
112.59	105,43	126,03	116,77	114,24	111,70		

Table 2. Normalized results of two different sets of BPA solutions (value/mean of the controls*100).

C_{BPA} mg/mL	1st BPA samples			Mean	SD
0	110,65	105,43	126,48	114,19	10,96
2.5	101,84	91,09	118,87	103,93	14,01
5	87,51	91,99	113,49	97,66	13,89
7,5	95,12	99,30	115,13	103,19	10,55
10	90,79	88,40	123,05	100,75	19,35
20	101,24	96,47	121,11	106,27	13,07
30	104,98	113,04	123,64	113,89	9,36
C_{BPA} mg/mL	1st BPA samples			Mean	SD
0	106,84	114,98	138,43	120,08	16,40
2.5	99,30	102,74	130,66	110,90	17,20
5	97,36	87,66	116,77	100,60	14,83
7,5	98,11	80,94	124,54	101,19	21,97
10	99,30	94,82	139,62	111,25	24,67
20	104,68	100,95	142.46	116,03	22.9 7
30	117,97	132,75	150,07	133,60	16,07

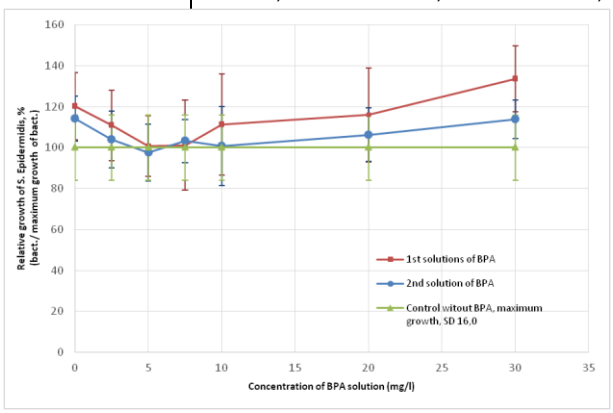


Figure 1. The toxicity results of BPA solutions using *S. epidermidis*.

Table 3. Normalized toxicity results of the experiments using *S. epidermidis* (value/mean of the controls). Value in the table is the mean of all three measurements. Because 0-sample was not taken (the first one only after 5 minutes), the value used is BPA (30mg/L), which does not contain H_2O_2.

	K_186.01_7.5		**E2_100.63_7.5_4**		**D4_161_10.0**		**I_100.63_11.04**	
T(min)	mean	SD	mean	SD	mean	SD	mean	SD
0	1,237	0,160	1,237	0,160	1,237	0,160	1,237	0,160
5	1,260	0,092	1,801	0,115	1,309	0,038	1.5 90	0,086
10	1,310	0,103	1,883	0,267	0,962	0,115	1,319	0,178
20	1.5 19	0,197	1,724	0,008	1,020	0,236	1,459	0,432
30	1,188	0,140	1,657	0,299	1,306	0,232	1,437	0,300
45	1,243	0,195	1,603	0,366	1.5 84	0,358	1,334	0,194
60	1,620	0,215	1,904	0,106	1,669	0,489	1,637	0,561
75	1,668	0,181	1,633	0,426	1,621	0,281	1,637	0,271
90	1,775	0,125	1,843	0,073	1.5 73	0,263	1,869	0,106

	B1_40.25_10.0		**H_100.63_3.96**		**C3_161_5.0**		**A2_40.25_5.0**	
T(min)	mean	SD	mean	SD	mean	SD	mean	SD
0	1,237	0,160	1,237	0,160	1,237	0,160	1,237	0,160
5	1,363	0,393	1,220	0,138	1.5 91	0,252	1,727	0,058
10	1,286	0,353	0,994	0,106	1,158	0,064	1,687	0,130
20	1,267	0,398	1.5 73	0,339	1.5 47	0,171	1,699	0,112
30	1,173	0,326	1.5 94	0,246	1,284	0,279	1,600	0,388
45	1,110	0,095	1,418	0,265	1,420	0,291	1,638	0,262
60	1,466	0,453	1,626	0,138	1.5 76	0,064	1,827	0,182
75			1,708	0,161	1.5 09	0,390	1,752	0,145
90			1,723	0,046	1,677	0,208	1,730	0,278

	B2_40.25_10.0		**D3_161_10.0**		**C2_161_5.0**		**F_100.63_7.5_2**	
T(min)	mean	SD	mean	SD	mean	SD	mean	SD
0	1,237	0,160	1,237	0,160	1,237	0,160	1,237	0,160
5	1,919	0,137	1,143	0,023	1,199	0,071	1,681	0,233
10	1,770	0,098	1,405	0,245	1,059	0,082	1,757	0,454
20	1,784	0,246	1.5 71	0,476	1,093	0,064	1.5 89	0,063
30	1,664	0,540	1,888	0,029	1,209	0,164	1,409	0,366
45	1,697	0,092	1,882	0,095	1,179	0,096	1.5 80	0,310
60	1,863	0,144	1,701	0,081	1.5 47	0,056	1,364	0,017
75	1,492	0,219	1,742	0,080	1,341	0,278	1,364	0,254
90	2.043	0,262	1,902	0,239	1.5 81	0,357	1,736	0,052

	D2_161_10.0		**B3_40.25_10.0**		**E_100.63_7.5**		**J_15.24_7.5**	
T(min)	mean	SD	mean	SD	mean	SD	mean	SD
0	1,237	0,160	1,237	0,160	1,237	0,160	1,237	0,160
5	1,864	0,109	1,690	0,177	1,429	0,078	1,630	0,095
10	1,762	0,346	1,697	0,071	1,431	0,444	1,886	0,330
20	1.5 33	0,547	1,897	0,105	1,409	0,268	1.5 59	0,334
30	1,777	0,459	1,652	0,412	1,185	0,159	1,710	0,256
45	1,462	0,457	1,323	0,095	0,976	0,154	1.5 30	0,331
60	1,347	0,494	1,673	0,249	1,193	0,483	1,619	0,268
75	1,839	0,491	1,825	0,067				
90	1,610	0,109	1,736	0,165				

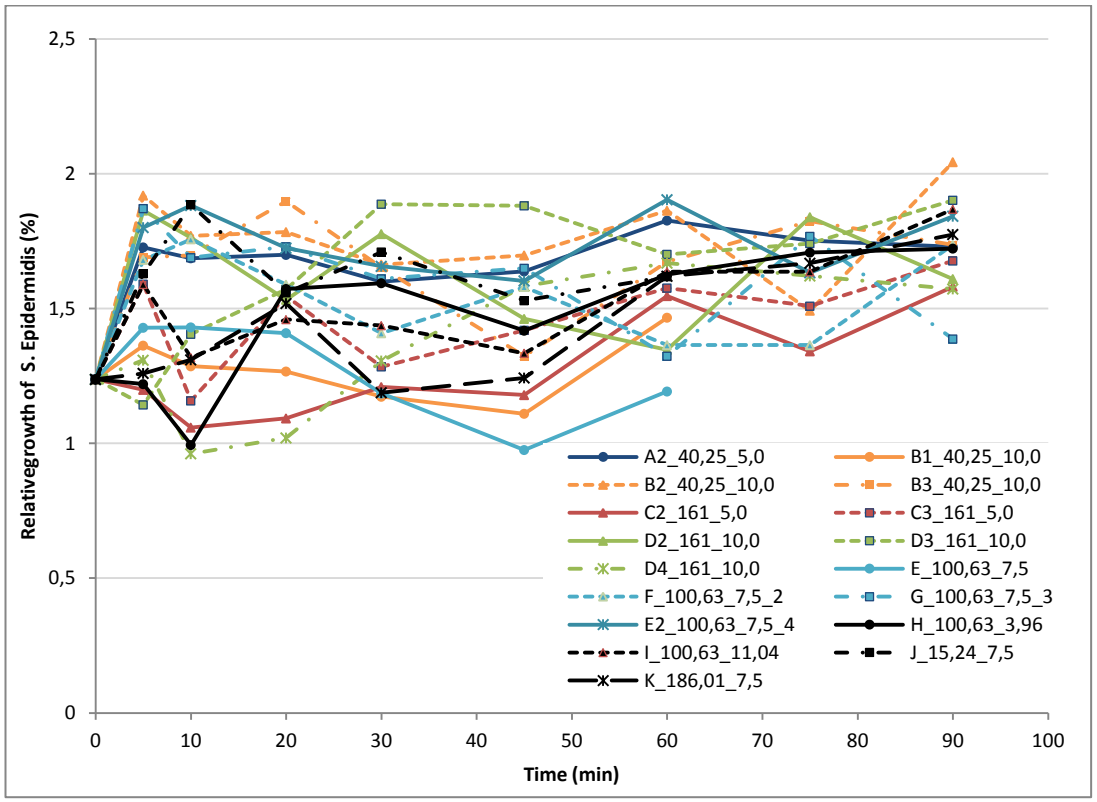
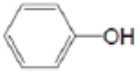
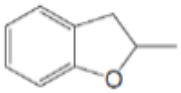
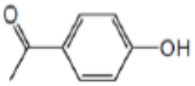
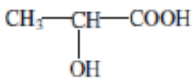
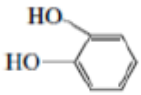
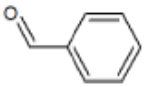
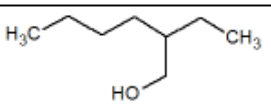


Figure 2. The relative growth of the *S. epidermidis*. This means bacteria growth divided by the maximum growth of the bacteria (= the mean of controls). It should be noted that 0-value is the value of BPA [30mg/L] without H_2O_2, because samples were not taken in the beginning of the experiment (the first sample was taken after 5 minutes).

Appendix 15

Toxic by-products of BPA found during the photo-Fenton reaction

Table 1. Intermediate compounds Navarro had studied (2013, 75).

Nº	Nom	CAS	Estructura	Con. (% ref Co_{BPA})
1 (K,P,R)	bisfenol A	80-05-7		
2 (K,P)	p-benzoquinona	106-51-4		0,31
3 (K,P,R)	fenol	108-95-2		0,46
4 (K,P)	p-bencenodiol	123-31-9		9,7
5 (K)	2-metil-2,3-dihidro-1-benzofurà	1746-11-8		
6 (P,R)	4-acetilfenol	99-93-4		4,65
7 (P)	Àcid 2-hidroxipropanoic	50-21-5		1,55
8 (P)	o-benzenodiol	120-80-9		1,4
9 (R)	Benzoaldehid	100-52-7		
10 (R)	1-hexanol-2-etil	104-76-7		
11 (R)	4,4'-dihidroxibenzofenona	611-99-4